INVESTIGATIONS ON FOOT ROT (*Fusarium moniliforme* SHELD.) OF BASMATI RICE WITH REFERENCE TO SURVIVAL AND VARIABILITY OF PATHOGEN AND HOST-RESISTANCE

Dissertation

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY
in
PLANT PATHOLOGY
(Minor Subject: Plant Breeding and Genetics)

By

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(L-2012-A-37-D)

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CERTIFICATE - I

This is to certify that the dissertation entitled, **"Investigations on foot rot (*Fusarium moniliforme* Sheld.) of basmati rice with reference to survival and variability of pathogen and host-resistance"** submitted for the degree of **Ph.D.**, in the subject of **Plant Pathology** (Minor subject: **Plant Breeding and Genetics**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Anita Puyam (L-2012-A-37-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that the dissertation entitled, **"Investigations on foot rot (*Fusarium moniliforme* Sheld.) of Basmati Rice with reference to survival and variability of pathogen and host-resistance"** submitted by **Anita Puyam** (Admission No. **L-2012-A-37-D**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.,** in the subject of **Plant Pathology** (Minor subject: **Plant Breeding and Genetics**) has been approved by the Student's Advisory Committee after an oral examination on the same.

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Place: Ludhiana

Date: ***(Anita Puyam)***

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ABSTRACT

The foot rot (bakanae) disease of rice caused by *Fusarium moniliforme* (syn. *F. verticilloides*) has become a serious bottleneck in the successful cultivation of basmati rice in Punjab and its adjoining areas. Studies of cultural characteristics of *F. moniliforme* on Potato dextrose agar (PDA), Soil Extract Agar (SEA) and Spezieller Nahrstoffarmer Agar (SNA) showed that all the 38 isolates produced whitish to pinkish color on the surface with dense and fluffy growth on both PDA and SEA, and moderate but not fluffy on SNA. The best growth was observed on PDA, followed by SEA and SNA. The growth rate of different isolates varied between 12.28-25.86-mm/ 48 hrs on PDA, between 9.09-19.59 mm/ 48 hrs on SEA and between 5.97- 16.44 mm/ 48hrs on SNA media. The size of the spores varied in the range of (6.8-9.8) X (2.9-6.0) µm for micro-conidia with no septa and (24.23-62.97) X (2.90-4.27) µm for macro-conidia having 3-5 septa. Micro-conidia were formed in chains on water agar with KCl and none of the isolates produced chlamydospores. DNA of 35 out of the 38 isolates was amplified with the set of primers VERT1 and VERT2 specific for *F. verticilloides* and gave a single amplicon of 800bp. All the isolates showed variable production of gibberellic and fusaric acid. All 38 isolates produced gibberellic acid whereas fusaric acid was produced by 30 isolates. Statistical analysis showed that the production of fusaric acid significantly correlated positively with the number of stunted plants and gibberellic acid production positively correlated with the number of elongated plants. Molecular studies using Simple Sequence Repeats (SSR) primers revealed 102 alleles using 27 primers. Out of 27 markers, all of the markers showed polymorphism with least polymorphism shown by primer (8H01) and no polymorphism by primer 2H06. Maximum number of alleles was produced by the primer 5H09. Polymorphic Information Content (PIC) values varied from 0.10-0.89 with an average of 0.46. Eleven SSR primers revealed PIC values higher than the average. The primers 7H05, 11H01, 5H09, 3H19, 10H09, 10H01, 9H05, 1H02, 2H17, 2H15, 3H02, 11H03 and 10H07 showed PIC values equal to or higher than 0.50. The genetic dissimilarity index ranged from 25 to 75%. The variability in pathogen, its survival and identification of resistant sources will help to formulate effective strategies for management of foot rot. Investigations on survival of *Fusarium moniliforme* in soil revealed that the pathogen doesn't survive in soil up to next season. Out of nine weeds evaluated against foot rot under artificial inoculation conditions in field, none of the weeds was infested. Out of 134 basmati germplasm lines screened under artificial inoculation field conditions, 24 lines were moderately susceptible, 4 lines were susceptible, 4 were highly susceptible, 27 were moderately resistant, 7 lines were resistant and 5 lines were highly resistant.

Key words: Foot rot, *Fusarium moniliforme,* Gibberellic, Fusaric, Simple Sequence Repeats, variability, Survival, Resistant

Signature of Major Advisor **Signature of the student**

ਖੋਜ ਪ੍ਰਬੰਧ ਦਾ ਸਿਰਲੇਖ	:	ਰੋਗਾਣੂ ਦੀ ਜਿਉਂਦੇ ਰਹਿਣ ਦੀ ਸਮਰੱਥਾ ਅਤੇ ਵਿਭਿੰਨਤਾ ਅਤੇ ਹੋਸਟ ਦੀ ਟਾਕਰਾ ਕਰਨ ਦੀ ਸਮਰੱਥਾ ਦੇ ਲਿਹਾਜ਼ ਨਾਲ ਬਾਸਮਤੀ ਝੋਨੇ ਵਿੱਚ ਪੈਰ ਗਲਣ *Fusarium moniliforme* (syn. *F. verticilloides*) ਰੋਗ ਦਾ ਅਧਿਐਨ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਅਨੀਤਾ ਪੁਆਮ (ਐੱਲ-2012-ਏ-37-ਡੀ
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ਨਿਮਨ ਵਿਸ਼ਾ	:	ਪੌਦਾ ਪ੍ਰਜਨਨ ਅਤੇ ਜੈਨੇਟਕਿਸ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਂ ਅਤੇ ਅਹੁੱਦਾ	:	ਡਾ. ਪੀ.ਪੀ.ਐੱਸ. ਪੱਨੂੰ ਸੀਨੀਅਰ ਪਲਾਂਟਪੈਥੋਲੋਜੀਜ
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ਸਾਰ

ਝੋਨੇ ਵਿੱਚ *Fusarium moniliforme* (syn. *F. verticilloides*) ਦੁਆਰਾ ਹੋਣਾ ਵਾਲਾ ਪੈਰ ਗਲਣ (bakanae) ਦਾ ਰੋਗ ਪੰਜਾਬ ਅਤੇ ਇਸੇ ਲਾਗਲੇ ਖੇਤਰਾਂ ਵਿੱਚ ਬਾਸਮਤੀ ਦੀ ਕਾਸ਼ਤ ਦੀ ਸਫਲਤਾ ਵਿੱਚ ਇੱਕ ਗੰਭੀਰ ਸਮੱਸਿਆ ਹੈ। ਆਲੂ ਡੈਕਸਟ੍ਰੋਜ਼ ਅਗਾਰ (PDA), ਮਿੱਟੀ ਨਿਚੋੜ ਅਗਾਰ (SEA) ਅਤੇ Spezieller Nahrstoffarmer ਅਗਾਰ (SNA) ਉਪਰ *F. moniliforme* ਦੇ ਕਲਚਰ ਗੁਣਾਂ ਦੇ ਅਧਿਐਨ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਸਾਰੇ ਦੇ ਸਾਰੇ 38 ਨਿਖੇੜਕਾਂ ਨੇ PDA ਅਤੇ SEA ਦੀ ਸਤ੍ਹਾ ਉਪਰ ਸੰਘਣੀਆਂ ਅਤੇ ਰੂੰ-ਨੁਮਾ ਅਤੇ SNA ਉਪਰ ਦਰਮਿਆਨੀ ਪਰ ਗੈਰ-ਰੂੰਨੁਮਾ ਸਫੈਦ ਤੋਂ ਗੁਲਾਬੀ ਰੰਗ ਦੀਆਂ ਕਲੋਨੀਆਂ ਵਿਕਸਿਤ ਕੀਤੀਆਂ। ਸਭ ਤੋਂ ਵਧੀਆ ਵਿਕਾਸ PDA ਉਪਰ ਹੋਇਆ ਅਤੇ ਇਸ ਉਪਰੰਤ SEA ਅਤੇ SNA ਵਿੱਚ। PDA, SEA ਅਤੇ SNA ਮੀਡੀਆ ਉਪਰ ਵੱਖੋ-ਵੱਖਰੇ ਨਿਖੇੜਕਾਂ ਦੀ ਵਿਕਾਸ ਦਰ ਕ੍ਰਮਵਾਰ 12.28-25.86 ਮਿ.ਮੀ ਪ੍ਰਤੀ 48 ਘੰਟੇ, 9.09-19.59 ਮਿ.ਮੀ ਪ੍ਰਤੀ 48 ਘੰਟੇ ਅਤੇ 5.97-16.44 ਮਿ.ਮੀ ਪ੍ਰਤੀ 48 ਘੰਟੇ ਦਰਜ ਕੀਤੀ ਗਈ। ਸੈਪਟਾ ਰਹਿਤ ਮਾਈਕ੍ਰੋ-ਕੋਨੀਡਾ ਉਪਰ ਬੀਜਾਣੂਆਂ ਦਾ ਆਕਾਰ (6.8-9.8) X (2.9-6.0) ਮਾਈਕ੍ਰੋ ਮੀਟਰ ਅਤੇ 3-5 ਸੈਪਟਾ ਵਾਲੇ ਮੈਕ੍ਰੋ-ਕੋਨੀਡੀਆ ਲਈ ਬੀਜਾਣੂਆਂ ਦਾ ਆਕਾਰ (24.23-62.97) X (2.90-4.27) ਮਾਈਕ੍ਰੋ ਮੀਟਰ ਦਰਜ ਕੀਤਾ ਗਿਆ। KCl ਨਾਲ ਜਲ ਅਗਾਰ ਉਪਰ ਛੋਟੇ-ਕੋਨੀਡੀਆ ਦੀ ਬਣਤਰ ਸੰਗਲ ਨੁਮਾ ਸੀ ਅਤੇ ਕਿਸੇ ਵੀ ਨਿਖੇੜਕ ਨੇ ਕੈਲਮਾਈਡੋਸਪ੍ਰੋਰ ਵਿਕਸਤ ਨਹੀਂ ਕੀਤੇ। *F. verticilloides* ਲਈ ਵਿਲੱਖਣ VERT1 ਅਤੇ VERT2 ਪ੍ਰਾਈਮਰਾਂ ਦੇ ਸਮੂਹ ਨਾਲ 38 ਨਿਖੇੜਕਾਂ ਵਿੱਚ 35 ਨਿਖੇੜਕਾਂ ਦੇ DNA ਕੀਤਾ ਗਿਆ ਜਿਸ ਤੋਂ 800 bp ਦਾ ਇਕਹਿਰਾ ਐਮਪਲੀਕੋਨ ਪ੍ਰਾਪਤ ਹੋਇਆ। ਸਾਰੇ ਦੇ ਸਾਰੇ ਨਿਖੇੜਕਾਂ ਨੇ ਵੱਖੋ-ਵੱਖਰੀ ਮਿਕਦਾਰ ਵਿੱਚ ਜ਼ਿਬਰੈਲਿਕ ਅਤੇ ਫੁਜ਼ੇਰਿਕ ਤੇਜਾਬ ਦਾ ਉਤਪਾਦਨ ਕੀਤਾ। ਸਾਰੇ ਦੇ ਸਾਰੇ 38 ਨਿਖੇੜਕਾਂ ਨੇ ਜ਼ਿਬਰੈਲਿਕ ਤੇਜਾਬ ਦਾ ਜਦੋਂਕਿ 30 ਨਿਖੇੜਕਾਂ ਨੇ ਫੁਜ਼ੇਰਿਕ ਤੇਜਾਬ ਦਾ ਉਤਪਾਦਨ ਕੀਤਾ। ਛੋਟੇ ਕੱਦ ਵਾਲੇ ਪੌਦਿਆਂ ਨਾਲ ਫੁਜ਼ੇਰਿਕ ਤੇਜਾਬ ਦੇ ਉਤਪਾਦਨ ਅਰਥਪੂਰਨ ਅਤੇ ਧਨਾਤਮਕ ਅਤੇ ਲੰਬੇ ਪੌਦਿਆਂ ਨਾਲ ਜ਼ਿਬਰੈਲਿਕ ਤੇਜਾਬ ਦੇ ਉਤਪਾਦਨ ਦਾ ਅਰਥਪੂਰਨ ਅਤੇ ਧਨਾਕਤਮ ਸਬੰਧ ਵੇਖਿਆ ਗਿਆ। SSR ਪ੍ਰਾਈਮਰਾਂ ਨਾਲ ਕੀਤੇ ਗਏ ਆਣਵਿਕ ਅਧਿਐਨ ਤੋਂ 27 ਪ੍ਰਾਈਮਰਾਂ ਨਾਲ 102 ਐਲੀਲਸ ਦਾ ਪਤਾ ਚੱਲਿਆ। 27 ਮਾਰਕਰਾਂ ਵਿੱਚੋਂ, ਸਾਰੇ ਦੇ ਸਾਰੇ ਮਾਰਕਰਾਂ ਨੇ ਬਹੁਰੂਪਤਾ ਵਿਖਾਈ, 8H01 ਪ੍ਰਾਈਮਰ ਨੇ ਸਭ ਤੋਂ ਘੱਟ ਅਤੇ 2H06 ਪ੍ਰਾਈਮਰ ਨੇ ਬਿਲਕੁਲ ਵੀ ਬਹੁਰੂਪਤਾ ਨਹੀਂ ਵਿਖਾਈ। 5H09 ਪ੍ਰਾਈਮਰ ਨੇ ਸਭ ਤੋਂ ਵਧੇਰੇ ਐਲੀਲਸ ਦਾ ਉਤਪਾਦਨ ਕੀਤਾ। ਬਹੁਰੂਪਤਾ ਜਾਣਕਾਰੀ ਪ੍ਰਕਰਣ (PIC) ਦੀਆਂ ਮਿਕਦਾਰਾਂ 0.10-0.89 ਤੱਕ ਦਰਜ ਕੀਤੀਆਂ ਗਈਆਂ ਅਤੇ ਇਹਨਾਂ ਦੀ ਔਸਤਨ ਮਿਕਦਾਰ 0.46 ਸੀ। ਗਿਆਰਾਂ SSR ਪ੍ਰਾਈਮਰਾਂ ਦੀ PIC ਮਿਕਦਾਰ ਔਸਤਨ ਮਿਕਦਾਰ ਤੋਂ ਵਧੇਰੇ ਸੀ। 7H05, 11H01, 5H09, 3H19, 10H09, 10H01, 9H05, 1H02, 2H17, 2H15, 3H02, 11H03 ਅਤੇ 10H07 ਪ੍ਰਾਈਮਰਾਂ ਦੀਆਂ PIC ਮਿਕਦਾਰਾਂ 0.50 ਦੇ ਬਰਾਬਰ ਜਾਂ ਇਸ ਤੋਂ ਵਧੇਰੇ ਸਨ। ਅਨੁਵਾਂਸ਼ਿਕੀ ਅਸਮਾਨਤਾ ਅੰਕ 25% ਤੋਂ 75% ਤੱਕ ਦਰਜ ਕੀਤਾ ਗਿਆ। ਰੋਗਾਣੂ ਵਿੱਚ ਵਿਭਿੰਨਤਾ, ਇਸਦੇ ਜਿਉਂਦੇ ਰਹਿਣ ਦੀ ਸਮਰੱਥਾ ਅਤੇ ਪ੍ਰਤੀਰੋਧਕਤਾ ਦੇ ਸਰੋਤਾਂ ਦੀ ਪਹਿਚਾਣ ਤੋਂ ਪੈਰ ਗਲਣ ਰੋਗ ਦੀ ਰੋਕਥਾਮ ਲਈ ਅਸਰਦਾਰ ਵਿਉਂਤਬੰਦੀ ਕਰਨ ਵਿੱਚ ਮਦਦ ਮਿਲੇਗੀ। ਮਿੱਟੀ ਵਿੱਚ *Fusarium moniliforme* ਦੀ ਜਿਉਂਦੇ ਰਹਿਣ ਦੀ ਸਮਰੱਥਾ ਦੇ ਅਧਿਐਨ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਇਹ ਰੋਗਾਣੂ ਮਿੱਟੀ ਵਿੱਚ ਅਗਲੇ ਮੌਸਮ ਤੱਕ ਜਿਉਂਦਾ ਨਹੀਂ ਰਹਿੰਦਾ। ਖੇਤ ਵਿੱਚ ਬਨਾਵਟੀ ਇਨੋਕੁਲੇਸ਼ਨ ਹਲਾਤਾਂ ਅਧੀਨ ਪੈਰ ਗਲਣ ਰੋਗ ਵਿਰੁੱਧ ਮੁਲਾਂਕਤ ਕੀਤੇ ਗਏ ਨੌਂ ਨਦੀਨਾ ਵਿੱਚੋਂ, ਕੋਈ ਵੀ ਨਦੀਨ ਇਸ ਰੋਗ ਤੋਂ ਪ੍ਰਭਾਵਤ ਨਹੀਂ ਹੋਇਆ। ਖੇਤ ਵਿੱਚ ਖੁਦ ਬਿਮਾਰੀ ਲਗਾ ਕੇ ਜਾਂਚੀਆਂ ਗਈਆਂ 134 ਬਾਸਮਤੀ ਜਰਮਪਲਾਜ਼ਮ ਲਈਨਾਂ ਵਿੱਚੋਂ, 24 ਲਾਈਨਾਂ ਦਰਮਿਆਨੇ ਪੱਧਰ ਤੱਕ ਸੰਵੇਦਨਸ਼ੀਲ, 4 ਲਾਈਨਾਂ ਸੰਵੇਦਨਸ਼ੀਲ, 4 ਅਤਿ ਸੰਵੇਨਸ਼ੀਲ, 27 ਦਰਮਿਆਨੇ ਪੱਧਰ ਤੱਕ ਪ੍ਰਤੀਰੋਧਕ, 7 ਲਾਈਨਾਂ ਪ੍ਰਤੀਰੋਧਕ ਅਤੇ 5 ਲਈਨਾਂ ਬਹੁਤ ਵਧੇਰੇ ਪ੍ਰਤੀਰੋਧਕ ਸਨ।

ਮੁੱਖ ਸ਼ਬਦ: ਪੈਰ ਗਲਣ ਦਾ ਰੋਗ, *Fusarium moniliforme*, ਜ਼ਿਬਰੈਲਿਕ, ਫੂਜ਼ੇਰਕਿ, ਐਸ.ਐਸ.ਆਰ., ਵਿਭਿੰਨਤਾ, ਜਿਉਂਦੇ ਰਹਿਣਾ, ਪ੍ਰਤੀਰੋਧਕ

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

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CHAPTER-I

INTRODUCTION

Rice is one of the major cereal crops grown in many parts of the world. It is mostly consumed in Asian countries and produced in more than 90% of the world (Singh *et al* 2013). India has the largest area in the world under rice cultivation with a total area of 43.52 million hectares, generating an annual production of 159.20 million tonnes and an average productivity of 3.59 t/ha of paddy during the year 2013 (FAO 2014). Presently, India exported 10.23 million tonnes of rice (both basmati and non basmati) and ranks second in rice production next to China. India has emerged as the leading exporter of rice. According to Punjab Marketing Board (PMB) data, 34.73 lakh tonnes basmati rice production has been recorded in Punjab during April 1, 2015 to March 31, 2016 (Anonymous 2016). Since April 2015 to January 2016, basmati rice export has been recorded at 30.73 lakh tonnes with a monthly average of 3.40 lakh tonnes.

Several abiotic and biotic factors constrained the production of rice crop (Singh *et al* 2012, Singh *et al* 2014). Foot rot (bakanae) disease, caused by *Fusarium moniliforme* Sheldon (synonyms: *F. verticilloides* (Sacc.) Nirenberg (Seifert 2003), *G. fujikuroi* Sawada mating population A, *Gibberella moniliformis* Wineland), emerged presently as a significantly important disease in all the countries where rice is grown (Venturini *et al* 2013). Based on morphological characters, many plant pathologists have reported *F. moniliforme* was the only species involved in causing foot rot disease of rice. In recent years, many researchers gave contradictory views in regard to the possibility of the involvement of other *Fusarium* species belonging to section *Liseola* in the production of foot rot symptoms (Amoah *et al* 1995, Desjardins *et al* 2000, Wulff *et al* 2010). Three mating populations MP-A (anamorph *F. verticilloides*), C (anamorph *F. fujikuroi*) and D (anamorph *F. proliferatum*) of the *G. fujikuroi* complex have been reported to associate with foot rot disease of rice (Bashyal and Aggarwal 2013, Gupta *et al* 2015). Predominantly, *F. fujikuroi* Nirenberg has been reported to involve in causing the foot rot disease of rice and were reported to be more virulent than other species (Gupta *et al* 2015). The teleomorph, *Gibberella fujikuroi*, has been reported in rice in China, Japan and Taiwan (Sun 1975). Typical symptoms of foot rot in rice plants are usually characterized with thin and elongated plants, the infected plants were taller than normal height of the other plants in seedbed and field; formation of root lesion which die before or after emergence of the seedlings; surviving plant at maturity formed empty or partially filled sterile grains. It is considered to be a high threat because it causes significant seedling mortality and yield loss. The disease is said to have been known in Japan since 1828 (Ou 1985). The disease is also known as *Fusarium* blight, white stalk, bakanae, foolish plant, elongation disease, fusariosis and foot rot in different countries. “Bakanae” is a Japanese word which means “thin noodle seedling”, “stupid rice crop”, “bad or naughty seedling” and

"foolish seedling", all referring to the abnormal elongation, caused by gibberellins produced by the pathogen upon infection of the host (Sun and Snyder 1981).

Among the major fungal diseases of rice including blast, sheath blight, brown spot and sheath rot, foot rot disease is one of the important biotic constraints in production of rice (Sharma and Thind 2007). It is continuously increasing every year especially in basmati rice (Anonymous 2014). Recent changes in cropping pattern and climate aggravated the disease even in non-traditional rice-growing areas. In India, foot rot disease incidence has been rising considerably and reported to cause substantial losses in grain yield. New methods of seedling raising, intensive cultivation of susceptible cultivars and poor management strategies contributed to high disease incidence (Bashyal *et al* 2015). Moderate to severe yield losses ranging from 15-25 percent have been reported from, Assam, Eastern U.P., Andhra Pradesh, Tamil Nadu, Punjab and Haryana (Sunder and Satyabir 1998b, Pannu *et al* 2012). Disease incidence on different basmati aromatic rice cultivars was recorded from 2.1 - 3.2 % in Uttarakhand, 1.2 - 11.7 % in Uttar Pradesh, 2.1 - 2.8 % in Haryana, 10.5 - 40.0% in Punjab, 1.8 - 8.7 % in Bihar and 2.4 - 13.6 % in Rajasthan (Gupta *et al* 2014). During the past 4-5 years, area under basmati rice has been increased due to its export potential. At present, basmati varieties occupies about 20 per cent of area under rice cultivation in Punjab. Basmati rice has the special status both in domestic and international market due to its immense export potential and excellent cooking qualities. During recent years, foot rot disease has become more prominent in India as an important disease of rice, mostly in basmati rice (Pannu *et al* 2012). Earlier the basmati rice cultivation was confined to sub-mountainous districts of the Punjab state however with the introduction of high yielding cultivars its cultivation has been started in all the districts of the state. This increases the concerned for the disease. Many factors including cultural practices, climate and host susceptibility contribute to the progressed of the disease (Parsons and Munkvold 2010). But, till date, the role of genetic variability of *F. moniliforme* in disease incidence has not been clearly defined (Venturini *et al* 2013). Information on *F. moniliforme* population genetics is limited with other than the two studies reported from Argentina (Reynoso *et al* 2009, Cumagun *et al* 2009). Little information is available on the population dynamics and diversity of *F. moniliforme* isolates under Punjab conditions. However, several workers (Amoah *et al* 1996, Al-Amodi 2006) in other parts of the world have demonstrated morphological, pathological and molecular variability of *F. moniliforme* isolates. Wulff *et al* 2010 reported pathogenicity, toxigenicity and genetic diversity of *Gibberella fujikuroi* species complex (GFSC) strains isolated from nine Asian and African countries.

In recent years, chemotaxonomic studies have revealed a specific profile of secondary metabolites in *Fusarium* species (Thrane 2001, Zainudin *et al* 2008b). *Fusarium* species in *Gibberella fujikuroi* species complex (GFSC) produced a number of secondary metabolites

(SMs) such as gibberellins (Yabuta *et al* 1937), fumonisin B1 and B2 produced by *F. proliferatum* and *F. verticillioides* (Marasas *et al* 1986, Desjardins *et al* 2000), fusaric acid (Bacon and Hinton 1996), moniliformine (Desjardins *et al* 2000), beauvericin and fusaproliferin (Reynoso *et al* 2004). They are responsible for pathogenicity and disease production of *G. fujikuroi* species complex (Ilija *et al* 2009), which is governed by the ratio of toxins and growth regulators (Amoah *et al* 1995, Zainudin *et al* 2008a). *Fusarium moniliforme* is known to produce fusaric acid (FA) and gibberellic acid (GA) (Zainudin *et al* 2008a). Gibberellic acid is a diterpenoid, first isolated from the fungus *Gibberella fujikuroi* as a super-elongation-promoting hormone. The species used different GA biosynthetic intermediates to produce GA_3 different from those in plants. Fusaric acid is a major toxin secreted by all *Fusarium* spp. Both GA and FA have a profound role in the production of elongation, stunting symptoms respectively, promoting pathogenicity. Different *Fusarium* species or different members of mating populations produce variable amount of GA and FA but not all the species causing foot rot disease produce FA (Kaur *et al* 2014). There is a scarcity of knowledge on the investigations of bio-chemical properties and their role in foot rot disease. Although several researchers have elucidated the host-pathogen interactions of *F. fujikuroi* and rice, informations on the roles of GA in pathogen growth and disease development in the host are limited (Hwang *et al* 2013).

In the present study, the pathogenic, bio-chemical, molecular and genetic diversity among Punjab populations of *F. moniliforme* (syn. *F. verticilloides*) has been investigated. Considering the importance of the disease under Punjab condition, identification and study of diversity of the pathogen is becoming very important. In depth study of the variability of the pathogen with reference to morphology, pathological, biochemical and molecular have been planned for study. To formulate proper management strategies, study of the biology of the pathogen with regard to their survivability and evaluation of different germplasm lines have been planned. Keeping these in view, the investigations have been conducted and the following objectives have been planned for study:

- To study morphological, pathological, bio-chemical and genetic variability in different isolates.
- To study the survival of *Fusarium moniliforme* in soil.
- To evaluate different genotypes of basmati rice against *Fusarium moniliforme.*

CHAPTER-II

REVIEW OF LITERATURE

2.1 Geographical distribution and economic importance

The foot rot fungus *F. fujikuroi* is widely distributed in temperate as well as tropical environment and throughout the world where rice is grown (Gupta *et al* 2015). Economically, the disease caused significant losses of approximately 20% of the rice cultivated areas in Asia (Cumagun *et al* 2015). But, it has never been observed in the rice-growing area of South-eastern Australia (Lanoiselet 2008). However, Heaton and Morschel (1965) reported *Fusarium* foot rot disease in the Northern Territory, Australia. In recent years, foot rot disease has been spreading and being observed in newer regions of Asia. In Thailand, Japan, China, Bangladesh, Pakistan and Nepal, foot rot disease has been gaining a major concerned since last decade. In India, 100% prevalence of the disease has been observed in all states *viz*., Uttarakhand, Uttar Pradesh, Haryana, Punjab, Bihar and Rajasthan (Gupta *et al* 2014). In Haryana, foot rot disease was first recorded during *Kharif* in 1988 on both groups of paddy i.e., scented tall varieties and high yielding dwarf (Sunder *et al* 1997). In Khanna district of Punjab, up to 40 % of the disease incidence was recorded in susceptible cultivar Pusa 1509. However, during the year 2014, overall disease incidence of 15-20 % was observed in the state (Gupta *et al* 2014).

2.2 Yield losses

The disease causes substantial losses in grain yield. The lowest disease incidence (4.17%) caused 3.4 per cent grain yield loss while the highest disease incidence (96.25%) caused the loss in yield of 95.45 per cent (Sunder *et al* 1997). The fungus affects rice crop in North America, Africa and Asia. In cases of epidemics, grain yield losses may reach up to 20% or more. Estimated crop losses of 15% in Thailand, 20% to 50% in Japan and 3.7% in India have been reported by the International Rice Research Institute in a 2003 publication (Suparyono *et al* 2009). In India, foot rot disease incidence has been increasing considerably and moderate to severe yield losses ranging from 15-25 percent have been reported from Assam, Eastern U.P., Tamil Nadu, Andhra Pradesh, Punjab and Haryana (Sunder *et al* 1998a, Pannu *et al* 2012). Particularly, the prevalence and incidence of the disease has been observed on basmati rice cultivars (Gupta *et al* 2014, Bashyal *et al* 2015). From 2008-2014 surveys in Northern states of India, the foot rot of disease incidence were reported at the ranged of 1.2-40 percent, particularly in basmati rice cultivars (Gupta *et al* 2014).

Apart from yield losses, Bashyal and Aggarwal (2013) also reported the foot rot pathogen associated with seed quality of different cultivars of basmati rice at the highest percentage (1-24%). The disease is particularly devastating in export quality scented rice varieties which earn substantial foreign exchange and is known to cause 3-95.4% loss in

different parts of the world (Singh and Sunder 2012). Therefore, the disease is a major concern in all the foot rot affected rice growing regions of India and has become an alarmed threat for sustainable rice production in other parts of the world where rice are grown. The disease has become an economically important in basmati rice in Punjab, during the last one decade. The disease has become a serious bottleneck in the successful cultivation of basmati in Punjab (Pannu *et al* 2013).

2.3 Causal organism of the disease

Fusarium heterosporum Nees (Hori 1898) was first identified as the causal organism of foot rot (bakanae) and *Lisea fujikuroi* was described as its teleomorph (Sawada 1917). There has been conflicting views among plant pathologists regarding the nomenclature of foot rot fungus. The imperfect stage of the fungus was described as *Fusarium moniliforme* (Sheldon) and the perfect stage as *Gibberella fujikuroi* (Sawada) Wr. (Wollenweber 1935). The terms '*Fujikuroi'* and 'Saw' in *G. fujikuroi* (Sawada) Wr. were derived from the name of two famous plant pathologists from Japan, Yosaburo Fujikuro and Kenkichi Sawada (Watanabe and Umehara 1997). This organism was later transferred to *Gibberella* as *G. fujikuroi* (Sawada) Ito (Ito 1931). *G. fujikuroi* (Sawada) Ito is a polytypic species complex constituting *G. fujikuroi* species complex (GFSC) with anamorphs in *Fusarium* species from section *Liseola* (Nirenberg and O'Donnell 1998, Leslie and Summerell 2006). The species constituting GFSC *viz., F. verticillioides* (Sacc.) Nirenberg (Nirenberg and O'Donnell 1998, Leslie and Summerell 2006), *F. proliferatum* (Mats.) Nirenberg (Amoah *et al* 1995, Desjardins *et al* 2000) and *F. fujikuroi* Nirenberg (Leslie and Summerell 2006) has been associated with foot rot (Nirenberg and O'Donnell 1998, Leslie and Summerell 2006). However, there are different views on whether all three species were associated with the incidence of the foot rot disease or *F. verticillioides* and *F. proliferatum* were present only as saprophytes (Wulff *et al* 2010). However, in Iran foot rot is known to be caused by *F. proliferatum* var. *proliferatum* (Matsushima) Nirenberg Nirenberg ex Gerlach & Nirenberg while in China *F. moniliforme* var. *zhejiangensis* has been identified as predominant pathogen associated with the disease (Singh and Sunder 1997). In a recent study, *F. verticilloides* and *F. proliferatum* were reported to be potential pathogens of rice causing bakanae (Wulff *et al* 2010, Young-Ah *et al* 2013). *F. concentricum* has also been reported to cause typical symptoms of foot rot disease (Young-Ah *et al* 2013).

2.4 Biology of *Fusarium moniliforme*

Presently, four species complex in plant pathogenic *Fusarium* has been reported: *Fusarium fujikuroi* complex; *Fusarium graminearum* complex; *Fusarium solani* complex and *Fusarium oxysporum* complex. *Fusarium moniliforme* belongs to *Fusarium fujikuroi* species complex (FFSC). These species induce foot rot disease in rice, ear rot in maize, pitch canker in pine. The species were reported to contaminate corn and other cereals with fumonisin

mycotoxins. Morphologically, *F. moniliforme* Sheld. belonged to Synder and Hansen's nine species system (Snyder and Hansen 1940) and Wollenweber and Reinking's section *Liseola* (Wollenweber and Reinking 1935). Biologically**,** thirteen biological species has been identified within the FFSC (Lima *et al* 2012) and phylogenetic analyses using several genes including β-tubulin and calmodulin revealed 46 species in the *G. fujikuroi complex* (O'Donnell *et al* 2013).Thirteen biological species are as follows:

1. *F.verticillioides* (Sacc.) Nirenberg,
2. *F. sacchari* (E.J. Butler & Hafiz Khan) W. Gams,
3. *F. fujikuroi* Nirenberg,
4. *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg,
5. *F. subglutinans* (Wollenw. & Reinking) P.E.Nelson, Toussoun & Marasas,
6. *F. thapsinum* Klittich, J.F. Leslie, P.E. Nelson & Marasas,
7. *F. nygamai* L.W. Burgess & Trimboli,
8. *F. circinatum* Nirenberg & O'Donnell,
9. *F. konzum* Zeller, Summerell & J.F. Leslie,
10. *F. xylarioides* Steyaert,
11. *F. musae* Van Hove, Waalwijk, Logrieco & Ant. Moretti,
12. *F. temperatum* Scaufl. & Munaut., and
13. *F. tupiense* Lima, Pfenning & Leslie.

Fusarium (Hypocreals, Ascomycota) is a taxonomically difficult genus. Under different environmental conditions, the species are highly variable and this has led taxonomists to consider some special criteria for the classification of the species (Booth 1975). Factors used for categorization and classification were variable growth (Thakur 1974), based on gibberellic acid production (Sunder 1995) and morphological characteristics i.e., their growth rate, pigmentation of culture and presence or absence of micro-conidial chain (Zainudin *et al* 2008a). However, identification based on morphological features is less reliable, thus the identification of different *Fusarium* species requires the characterization of their bio-chemical, physiological and molecular characters (Ismail *et al* 2013).

Morphologically, hyphae of *Fusarium moniliforme* are hyaline, branched and septate (Ilija *et al* 2009). The pathogen produces two types of spores: micro-conidia and macro-conidia. Micro-conidia are club or oval shaped with a flattened base and 0 to 1-septate (Ilija *et al* 2009, Pradeep *et al* 2013). Conidiogenous cells may be monophialides and polyphialides (Leslie and Summerell 2006) and are produced abundantly. The micro-conidia are, more or less, agglutinated in chains and remain joined or cut off in false heads (Ilija *et al* 2009). Macro-conidia are slightly sickle-shaped and delicate, relatively slender or almost straight with no significant curvature (Leslie and Summerell 2006, Ilija *et al* 2009). These are narrow at both ends and are occasionally somewhat bent into a hook at the apex and the basal cell is

distinctly or barely notched and number of septa varies from 3 to 5. These are abundantly formed in sporodochia (Ilija *et al* 2009). Chlamydospores are absent and chlamydospores-like or pseudochlamydospores may develop in the hyphae of the species (Leslie and Summerell 2006). Morphological characterization of *Fusarium* species have been done by many workers based on the observed morphological features like shapes, sizes and formation of macro-conidia and micro-conidia, formation of conidiophores and presence or absence of chlamydospores and pigmentation presence in culture (Zainudin *et al* 2008a, Rahjoo *et al* 2008, Hsuan *et al* 2010, Darvishnia 2013, Hwang *et al* 2013, Yadav *et al* 2014).

Mating populations (MPs) and bio-chemical metabolites particularly mycotoxins have also been reported as identifying characters for *Fusarium* to species and sub-species levels (Leslie and Summerell 2006, Zainudin *et al* 2008b). Chemotaxonomic findings revealed that each *Fusarium* species has a unique secondary metabolites profile. The studies on chemotaxonomic criteria may serve as supplements to morphological characteristics in delimitation of *Fusarium* species (Nelson *et al* 1993).Generally, different *Fusarium* species produced different secondary metabolites profiles *viz*., *F. fujikuroi* produce gibberellic acid (GA_3), (fumonisin) FB1 and moniliformin (MON) (Marasas *et al* 1986, Zainudin *et al* 2008b) whereas, *F. verticillioides* produce FB1 and little or no moniliformin (MON). The secondary metabolites information helped for assessing the risk of mycotoxin contamination in rice as well as for assisting in the identification of closely related species such as *F. fujikuroi*, *F. moniliforme* and *F. proliferatum.* Among other species of *Fusarium* associated with the foot rot disease of rice, *F. fujikuroi* has been regarded as the most virulent species causing the disease. It produces excessive gibberellin hormones which is responsible for internode elongation of bakanae infected plants (Zainudin *et al* 2008a). The other explanation for bakanae disease symptoms and excessive increase in plant height might be due to production of metabolites other than gibberellins. Desjardins *et al* (1995) also reported in maize crop that there is a correlation between the ability of the causal pathogen *F. verticillioides* to insert fumonisin *in-vivo* and the severity of maize seedling blight disease *in-vivo*.

The chemotaxonomic criteria have also been used to classify the fungus. Zainudin *et al* (2008b) identified thirty four isolates of *Fusarium* spp and among which only *F. fujikuroi* isolates were reported to produce GA_3. On the basis of this feature, they distinguished *F. fujikuroi* from the other four species of *Fusarium* isolated from bakanae-infected rice in Malaysia and Indonesia. In a similar case, strains of *F. proliferatum*, *F. sacchari*, *F. subglutinans* and *F. verticillioides* were reported to be unable to produce GA_3 and *F. fujikuroi* was the only species of *Fusarium* that can produce this plant growth hormone (Desjardins *et al* 2000). Malonick *et al* (2005) also reported that none other than *F. fujikuroi* and *F. konzum* produced gibberellic acids when different *Fusarium* spp were isolated from infected rice seedlings. They concluded that characteristic production of GA could be used as a main

physiological character in separating *F. fujikuroi* from other species. But, Desjardins *et al* (2000) reported *F. proliferatum* as a causal organism of foot rot disease which induced the production of gibberellins and caused elongation in infected plants. Proctor *et al* (2010) also reported five *F. proliferatum* strains that could produce gibberellins and hypothesized that their ability to produce gibberellins might be attributed to the transfer of gene responsible for gibberellic acid biosynthesis to the hybrid strain generated from *F. fujikuroi* and *F. proliferatum*. The hypothesis was further supported by the assumption that mixed reproduction and gene flow might be responsible for formation of new genotype (Cumagun *et al* 2015). Phylogenetically, *F. fujikuroi* and *F. proliferatum* are closely related species distinguished by mating types, chemotaxonomic criteria and molecular markers (Leslie and Summerell 2006).

Mating populations (MPs) is another criteria used to overcome these taxonomic difficulties and to distinguish the species. *Fusarium moniliforme* is a name that has been applied to any of six biological species (or mating populations) that share the teleomorph (sexual stage) *Gibberella fujikuroi* (Leslie 1995). Lately, the sexual stage formation has been used to distinguish biological species within this group or mating population (Hseieh *et al* 1977, Kuhlman 1982, Leslie *et al* 1995). Mating pattern within each of these MPs are heterothallic and governed by two alleles at a single mating-type locus. Members of the different MPs are not sexually fertile with one another but members under same MPs are sexually compatible. The three mating populations A, C and D that were reported to be associated with bakanae were identified from different geographical regions. Mating population C (MP-C) (anamorph *Fusarium fujikuroi*) was first identified in 1977 among isolates from Taiwan rice (Hsieh *et al* 1977). MP-A (anamorph *Fusarium verticillioides*) and MP-D (anamorph *Fusarium proliferatum*) were isolated from rice in Asia, Africa, USA and Australia (Voigt *et al* 1995, Desjardins *et al* 1997) and genetically inter-fertile MP-C strains from rice (Hseieh *et al* 1977, Kuhlman 1982). *G. fujikuroi*, *G. intermedia* and *G. moniliforme*, respectively were named as sexual stages of these pathogens (Gupta *et al* 2015). Group C is confined to the rice, while, the other groups have pine, rye, sugarcane, wheat, corn or sorghum, asparagus as their major hosts (Puhalla and Spieth 1985). Fourth mating population termed was identified and described as D by Kuhlman (1982) constituting of four varieties of *G. fujikuroi viz.*, *moniliformis, subglutinans, fujikuroi* and *intermedia* on the basis of mating population, ascospores, perithecia size, microconidial formations and phialide type. Nine biological species or mating populations designated as MP-A (*F. verticillioides*), MP-B (*F. sacchari*), MP-C (*F. fujikuroi*), MP-D (*F. proliferatum*), MP-E (*F. subglutinans*), MP-F (*F. thapsinum*), MP-G (F. *nygamai*), MP-H (*F. circinatum*) and MP-I (*F. konzum*) within the *G. fujikuroi* species complex in section *Liseola* has been reveal from genetic studies (Leslie and Summerell 2006).

2.5 Symptomalogy

This disease generally appears in nursery and upto 20-25 days after infection, however, it can be observed throughout the growing season. The plants are infected in seedling stage as well as after transplanting. The fungus infects plants through roots or crowns. It later becomes systemic, i.e. it grows within the plant and systemically infects the panicle. Diseased seedlings are pale yellow and often visibly taller than the healthy rice plants in seed bed and field. The infected seedlings can be severaltimes taller than healthy seedlings. Elongation is caused by gibberellins produced by the pathogen upon infection of the host (Bashyal *et al* 2012). Such seedlings die either before or after transplanting (Ilija *et al* 2009). If once infected, seedlings often progressively die from the seedling stage through to maturity. The micro-conidia and mycelium of the pathogen are found to be concentrated in the vascular bundles, particularly large pitted and xylem vessels. If the infected plants survive to heading, the panicles they produce are usually sterile. The fungus sporulates profusely on the stems of diseased plants near the water level, and after the water is drained, noticeable pink to white fungal growth appears at the base of the plants. This cottony growth produces masses of conidia which contaminate the outside of healthy seeds during harvest. Other symptoms include formation of pink sporodochia at palea and lemma junction of the damaged grains (Gupta *et al* 2015), formation of adventitious roots from lower nodes of stem has been reported from India (Thomas 1931). Whitish-pinkish fungal growth has also been reported from lower portion of the infected plants which contaminate the seed during harvesting (Surek and Gumustekin 1994).

2.6 Host range of the pathogen

Rice, barley, sorghum, maize, wheat, sugarcane, pine, rye and asparagus from Asia, South East Asia, United States and Africa have been reported as primary hosts of bakanae pathogen (Hseieh *et al* 1977, Kuhlman 1982, Puhalla and Spieth 1985, Wulff *et al* 2010, Petrovic *et al* 2013). Alternate hosts *viz*., proso millet, cowpea, banana, tomato, subabool, barnyard grass and early water grass may also serve as reservoir of inoculum in the field. Carter *et al* (2008) reported that *F. fujikuroi* survived in barnyard and early water grasses and the isolates collected from them scored positive for Koch's Postulates. However, no bakanae symptoms were observed (Carter *et al* 2008). This indicated the possibility of the grasses to be source of inoculum for the disease. *F. verticilloides* commonly infect a wide range of crops and plants throughout the world and no host specialization. It has been reported in maize, soybean, sugarcane, banana etc. Hirata *et al* (2001) reported the pathogenicity of *Fusarium verticillioides* strains to banana, soybean and maize and observed that all the isolates induced light to dark brown spots on wounded and non-wounded parts of inoculated healthy green banana fruit with whitish mycelial colonies and were often surrounded by halos. Krnjaj *et al* (2007) also reported the pathogenicity of 10 isolates of *Fusarium verticillioides* collected

from grain of wheat (five isolates) and maize (five isolates) under greenhouse conditions. Venturini *et al* (2013) reported *Fusarium verticillioides* populations in maize samples collected from Northern Italy and pathogenicity of all the isolates were tested on maize seeds by assessing the percentages of the seed germination and the percentage infection indexes of infected seed, coleoptile rot and radicle decay.

2.7 Survival of *Fusarium* in soil

Foot rot of rice is primarily a seed borne disease. The pathogen over-winters as spores on the coat of infested seeds. Sunder and Satyavir (1998b) reported that *F. verticillioides* survived for 26 months in grains of cultivar Haryana Gaurav and for 16 months in Taraori Basmati and Basmati 370. Kanajanasoon (1965) found that the fungus remain viable in seeds and infected plants only for 4-10 months at room temperature. Misra *et al* (1989) and Sunder and Satyavir (1998a) reported that the fungus survived in infected grains for 16-28 months. Diseased debris also serves as a primary source of inoculum. The fungus *F. verticillioides* has been reported to survive in plants debris for 10-82 weeks (Dodan *et al* 1994, Sunder *et al* 1997). Watanabe (1974) reported the role of soil borne inoculums in disease development. The fungus survives in soil through conidia, ascopores, thick walled hyphae (Sun 1975) and sclerotia (Sharma and Singh 1978). Nyvall and Kommedahl (1970) reported that thickened hyphae or mycelia fragments were the structures of survival. Sharma and Singh (1978) reported that in absence of native microflora in autoclaved soil hyphal propagules of *F. verticillioides* remained viable for 382 days while conidia were recovered only upto 215 days. The results of the experiment of Linddell and Burgess (1985) indicated that microconidia of *F. verticillioides* can survive for a long period under favourable conditions. Sunder and Satyavir (1998a) reported the survival of *F. moniliforme* in soil enriched with different nutrients and their combinations in which fungal recovery from stubble declined with the incubation period irrespective of amendments but none of nutrients at test concentrations affect the survival of the fungus after eight months of storage of stubble in soil. The fungus in infected stubbles survived for four months kept in soil under natural environmental conditions (Biswas and Das 2003, Pannu *et al* 2012). The pathogen inoculum is passed onto the next seasons in case when the rice is grown for consecutive years in the same field. Karov *et al* (2009) reported low foot rot incidence when clean seeds were used for sowing in fields from where the disease has been reported previously. It has been revealed that seed borne infection is more important as soil borne inoculums is reduced rapidly with passage of time (Singh and Sunder 1997).

2.8 Primary and secondary infection

Pathogen of foot rot is primarily a seed borne. Airborne spores or conidia contaminated and infected the seed during the flowering stage of the crop (Gupta *et al* 2015). Ascospores and conidia adhering to the seed act as primary source of inoculum germinate and

infect seedlings through the roots and crown (Sun 1975). Dissemination of the conidia by wind and water caused secondary infections in the rice field. Under favorable conditions, infected plants have the capacity to produce numerous conidia that subsequently infect neighboring plants at flowering stage. Infection may also take place through spores and mycelium that are left in the water used for soaking seeds to stimulate germination before sowing (Karov *et al* 2009). The plant becomes systemically infected except the panicle. The fungus can infect the seeds without producing visibly distinguishable symptoms and can even be isolated from healthy looking seeds.. Symptom development is influenced by the amount of inoculum present, the strain of the pathogen and the relative quantities of gibberellin and fusaric acid produced (Cumagun 2015) and the temperature (Lanoiselet 2008). Symptoms do not appear below 20ºC and the temperature 35ºC is optimum for disease development.

2.9 Isolation and culture

Isolations of the pathogen were made on common medium (Potato Dextrose Agar), selective medium (Peptone Pentachloronitrobenzene Agar), weak nutrient agar medium (Spezieller Nahrstoffarmer Agar) and natural substrate medium (Carnation Leaf Agar) for fungal culture (Zainudin *et al* 2008a, Pra *et al* 2010). Cultures grown on Spezieller Nahrstoffarmer Agar (SNA) media were often used for examining micro-conidia and chlamydospores. It promotes sporulation and good conidiogenous cell development (Leslie and Summerell 2006). Soil dilution method has also been used to isolate pathogen inoculum from soil around root zone of infected plants (Sharma and Singh 1978, Saremi and Farrokhi 2004, Saremi *et al* 2008). Various agar based media have been used as standards to grow cultures for the identification of *Fusarium* species. Potato dextrose agar (PDA), Spezieller Nahrstoffarmer Agar (SNA) and Carnation Leaf-piece Agar (CLA) have been used as the standard media for identification of *Fusarium* species (Leslie and Summerell 2006).

2.10 Morphological variability

Zainudin *et al* (2008a) collected thirty-four single-spore isolates and grown on PDA (potato dextrose agar) and CLA (carnation leaf agar) plates and identified as *F. fujikuroi, F. proliferatum, F. verticillioides, F. sacchari* and *F. subglutinans* based on morphological characteristics. Potato Dextrose Agar was used to measure growth rate and pigmentation of culture, while CLA was used to determine shape and size of macro-conidia and microconidia, conidiogenous cells and chlamydospore formation. Rahjoo *et al* (2008) identified a total of 191 *Fusarium* isolates based on morphological characters and using species-specific PCR assays. Pradeep *et al* (2013) analyzed eight soil samples and a total of 33 isolates were obtained and eight morphologically different color producing fungi were isolated, one of the isolate showed the ability to produce dark reddish brown pigment and identified as *Fusarium moniliforme* KUMBF1201. Ahangar *et al* (2014) reported mycelium of *Fusarium moniliforme* to be septate forming a dense coat of branching hyphae and had a width of 2-3µm.

Microconidiophores were short, simple arise laterally from hyphae and produce microconidia singly in chains. The micro-conidia were abundantly produced and single celled, ovoid or oblong, hyaline. Macroconidiophores with 2-3 apical phialides produced macroconidia. Macroconidia were 3-7 septate, slightly curved, equally bent at pointed ends. Yadav *et al* (2014) reported micro-conidial size of (5-12 X 1.5-2.5) µm with 0-1 septum and macroconidia of (25-60 X 2.5-4.0) µm with 3-7 septa. Ahangar *et al* (2014) also reported micro-conidial size of 4-10 x 1-2 µm and macro-conidial size of (20-50 x 2.0-3.5) µm. *F. moniliforme* produced pale orange color sporodochia but normally they are absent (Pradeep *et al* 2014). Hwang *et al* 2013 studied the colony morphology, colony diameter on PDA and its color (whitish pink with active sporulation). They reported the production of micro and macro-conidia and in particular, micro-conidia in chains on conidiophores, which is one of the important mycological characteristics of *F. fujikuroi*. Ahangar *et al* (2014) reported white cottony color colonies which later became pinkish in older cultures with reddish-brown to pink colored on the reverse.

F. proliferatum is similar to *F. verticillioides*, but the former species is distinguished by micro-conidial chain forming ability. The microconidial chains produced by *F. thapsinum* and *F. verticillioides* are generally longer than that produced by *F. proliferatum*. Ability to form pseudochlamydospores by *F. andiyazi* differentiates it from *F. verticilloides*. *F. thapsinum* and *F. verticillioides* can be reliably differentiated only by using mating tests or molecular markers, although many isolates of *F. thapsinum* produce yellow pigments that are unique to them (Leslie and Summerell 2006).

2.11 Radial Growth and Pigment Production of *Fusarium*

Fungi are source of stable colorants and rich in pigments. A number of media are used for isolation and identification of different groups of fungi. These culturing media influences the colony morphology and the vegetative growth, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Kumara and Rawal 2008). Environmental factors such as temperature, pH and water activity have a great influence on fungal growth and development. Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, shaking and inoculum size have great influence on the growth of pathogen (Bhattacharyya and Jha 2011). Effects of temperature, pH and carbon source on radial growth rate have been well assessed by Yadav *et al* (2014). Among the fungi producing stable colorants, *Fusarium moniliforme* has been known for producing variant colour of pigments. *Fusarium moniliforme* is a ubiquitous fungus distributed all over the world. The nature of the surface growth pattern either flat or cottony type were also influenced by the availability of nutrition, although it differs from species to species and strain to strain (Carlile 1995). Different culture media influenced the growth and

pigmentation of *Fusarium moniliforme* KUMBF1201 as reported by Pradeep *et al* (2013) on eight solid media (PDA, MA, RBA, OMA, YMA, CZA, SDA and NA). They revealed that maximum mycelial growth and requirements for carbon, nitrogen and sulfur and energy pigment production was best on PDA medium. *Fusarium moniliforme* forms white mycelia which becomes gray, violet or magenta with age. Pigmentation in the agar varies and ranges from no pigmentation or grayish orange in some isolates to dark violet or dark magenta (almost black), violet grey, in others (Leslie and Summerell 2006). Various carbon sources *viz.* sucrose, glucose and starch and agricultural residue such as rice husk and sugarcane bagasse were tested as sole carbon source for the growth of pathogen. Maximum growth was observed in medium containing rice husk and maximum sporulations were achieved in medium containing sugarcane bagasse (Yadav *et al* 2014). Pannu *et al* (2013) studied the growth of the fungus on different media among which the best being Potato Dextrose Agar and Nutrient Glucose Agar, followed by Richard's Agar medium and Czapek's Agar medium. The color of the fungal colony was pinkish white on Potato Dextrose Agar and Nutrient Glucose Agar, white-light red color on Richard's Agar medium and light pinkish white color on Czapek's Agar medium. The white color of the colony was observed on Water Agar (WA) and Spezieller Nahrstoffarmer Agar (SNA) medium. Ismail *et al* (2013) reported that growth rate, colony color on surface and reverse on Gycerol nitrate agar (G25N) and Czapek iprodione dichloran agar (CZID) were promising diagnostic criteria for separating species within sections. The ability to grow on tannin-sucrose agar could distinguish some species of sections *Liseola* and *Sporotrichiella*.

2.12 Pathogenic variability

Many phenotypic markers, such as pathogenicity, mycelial growth, conidial production and growth rate described the population dynamics in *F. verticilloides*. Among the various phenotypic properties for investigating variability of fungal population, study of pathogenicity has been the most suitable attribute for the study (Zhan *et al* 2007, Haque *et al* 2008). By considering pathogenicity as population marker previous studies revealed the difference between mating populations within *Gibberella fujikuroi* clade (Leslie *et al* 2005, Wulff *et al* 2010). Such differences helped to characterize *Fusarium* spp. associated with their host plants providing data on *Fusarium* variability and diversity (Venturini *et al* 2013). The pathogenic property has been quantified by several methods for example determining the disease severity (Zhan *et al* 2007), by assessing seed germination inhibition, seed colonization, determination of mating type and male/ hermaphrodite polymorphism (Venturini *et al* 2011) and radical decay; coleoptiles rot (Venturini *et al* 2013).

Nisikado and Matsumoto (1933) observed marked differences in pathogenicity of 66 strains of *G. fujikuroi* from rice as indicated by the degree of overgrowth as compared to the normal ones. Chin (1940) observed that some of the isolates caused dwarfing, some caused

elongation and others had no effect on the height of rice seedlings. Sunder and Satyavir (1998b) reported five virulence groups (VG-I–VG-V) of *F. moniliforme*. Disease suppression was found to be less or more dependent on the virulence of each isolate; different isolates showed different levels of virulence. Sharma and Bagga (2007) studied four isolates of *Fusarium moniliforme* based on mortality and symptoms produced by the pathogen including slender pale, elongation, stunting and overall incidence. All the isolates differed considerably in their pathogenic ability. Other workers also reported the variation in pathogenic behaviour. Zainudin *et al* (2008a) reported wide spread of bakanae disease in Peninsular Malaysia and three provinces of Indonesia with the range of disease severity from scale 1 to 5 and disease incidence from 0.5 to 12.5% during main growing seasons in 2004–2005. Kaur *et al* (2009) studied pathogenic variability of *F. moniliforme* and identified three isolates that varied considerably on the basis of pathogenicity. Amatullia *et al* (2010) classified a total of 121 isolates of *Fusarium* spp. obtained from naturally infected rice plants for pathogenicity on the basis of 0-100 scale as slightly pathogenic, not pathogenic and pathogenic. They reported that only *F. fujikuroi* showed bakanae symptoms and the other species *viz*., *F. proliferatum*, *F. verticilloides* failed to produce the symptoms. However, Young-Ah *et al* (2013) showed the involvement of other *Fusarium* spp *viz*. *F. fujikuroi*, *F. concentrum*, *F. proliferatum* and *F. verticillioides* in causation of the bakanae disease. They collected 110 seed samples from ten Asian countries, 33% of the samples from each country were infected with GFSC and the incidence (% infected seeds in a sample) of GFSC varied from 3% to 80%. Bashyal and Aggarwal (2013) also reported variation in pathogenicity and morphology of the *F. fujikuroi* isolates collected from rice seeds.

The pathogenic variability has also been studied in other hosts like maize, sugarcane etc. Krnjaj *et al* (2007) based on different parameters of the pathogenicity estimate (a scale for % of non-emerged plants, % of survived plants, plant vigour -the growth and dry weight of roots and epicotyls; and disease severity), it was determined that all *F. verticillioides* isolates expressed a different degree of pathogenicity. Mohammadi *et al* (2012) recovered seventy isolates in *Fusarium* section *Liseola* from sugarcane stalks infected with pokkah boeng disease including 50 isolates of *F. verticillioides* and all the isolates were pathogenic to sugarcane. Kaur *et al* (2014) indicated the different virulence spectra based on the pathological characterization of 56 isolates of *F. moniliforme* on both maize hybrids and inbred lines. They studied pathogenic and molecular characterization of *Fusarium moniliforme* Sheld, the incitant of *Fusarium* maize stalk rot in Punjab and on the basis of multivariate cluster analysis of pathogenicity data, seven clusters were reported, each representing a specific disease reaction to a particular maize hybrid/inbred line. Neither the mating types nor the female fertility were reported to influence the average pathogenicity of the fungal population (Venturini *et al* 2013).

The variation in pathogenicity has been attributed to the production of secondary metabolites produced by the pathogen as evident from the work of Nyvall (1999) and Abo-Elanga (2007). Nyvall (1999) reported that the fusaric acid and the gibberellic acid produced by *F. verticillioides* were responsible for stunting and elongation, respectively in many species of plants besides rice. Jimenez *et al* (2000) and Abo-Elanga (2007) also reported the involvement of fumonisin in determining the pathogenic capabilities of isolates.

2.13 Secondary Metabolites produced by *F. moniliforme*

Zainudin *et al* (2008b) first carried out the study on the secondary metabolites profiles such as productions of MON, FB1, GA_3 and FA by *Fusarium* spp in section *Liseola* isolated from rice in Malaysia and Indonesia. They reported that *F. fujikuroi* produced MON, FB1, GA3 and FA, *F. verticilloides* and *F.proliferatum*, produced all three except GA_3. *F. sacchari* and *F. subglutinans* didn't produce any of the four secondary metabolites. However, Desjardins *et al* (2000) reported *F. proliferatum* as a causal organism of bakanae disease which can induce elongation and production of gibberellins in infected plants. Fusaric acid and gibberellic acid produced by *F. verticillioides* caused stunting and elongation, respectively in many species of plants besides rice (Sood 1964, Young-Ah *et al* 2013, Brown *et al* 2014). *Fusarium* species within GFSC complex are able to produce mycotoxins that can affect animal and human health (Leslie *et al* 1992). Generally, *F. fujikuroi* can produce moniliformin, fumonisins and beauvericin, *F. verticillioides* can produce fumonisins, and *F. proliferatum* is able to synthesize fumonisins, fusaproliferin and beauvericin.

2.13.1 Gibberellic acid

Gibberellic acid **(**GA_3) is a growth promoting hormone that induces the elongation of plant. The role of GA_3 in pathogenicity has been attributed to the higher concentration of GA_3 than normal (Zainudin *et al* 2008b)**.** A positive correlation between GA_3 production in culture and bakanae has been observed (Sunder and Satyavir 1998b). Ma *et al* (2008) reported that *F. fujikuroi* usually produced large quantities of gibberellic acid (GA_3) in cultures and induced bakanae symptoms in experiments with artificial inoculation. They also found a significant correlation between the length of seedlings treated with GA_3 and bakanae injury. Thakur *et al* (1974) reported that the ratio of gibberellic acid and fusaric acid determine the type of symptoms either elongation/stunting of rice plants. Tochinai and Ishizuka (1934) found that presence of bakanae symptoms is limited to the actual period of contact with the toxin as the infected seedlings recovered after planting to an uninfected area.

2.13.2 Fusaric acid

Fusaric acid (FA) is one of the most widely distributed secondary metabolites produced by *Fusarium* (Zainudin *et al* 2008b). It is one of the oldest reported secondary metabolite. Chemically, it is 5-butylpicolinic acid, a mycotoxin with low to moderate toxicity to animals and humans, but with high phytotoxic properties (Niehaus *et al* 2014). Presence of

fusaric acid may give a presumptive indication of *Fusarium* contamination in food and feed grains (Bacon *et al* 1996). Knowing this property will help in assessing the risk of mycotoxins contamination in rice. Besides fusaric acid, *F. fujikuroi* also produces two fusaric acid-like derivatives: fusarinolic acid and 9, 10-dehydrofusaric acid. Fusaric acid was successfully detected as a yellow greenish fluorescent spotted on TLC plates. *G. fujikuroi* species complex (including *F. fujikuroi*, *F. verticillioides*, *F. subglutinans* and *F. proliferatum*) have previously been reported to produce FA (Burmeister *et al* 1985, Bacon *et al* 1996).

2.13.3 Fumonisins

They are series of structurally related sphingosine analogue toxin produced by *F. moniliforme* and other fungi (Jestoi *et al* 2004). They are cytotoxic and carcinogenic mycotoxins, mainly produced by *Fusarium verticilloides* (Sacc.) Nirenberg (previously known as *Fusarium moniliforme* Sheldon.).Twenty-eight structurally related fumonisin analogues have been reported, only three of them fumonisin B1 (FB1), B2 and B3 occurred abundantly (Ghiasian *et al* 2004). Maheshwar *et al* (2009) reported that out of the four *Fusarium* spp.; *F. verticilloides*, *F. proliferatum*, *F. anthophilum* and *F. graminearum*, only *F. verticilloides* was reported to produced fumonisin. Fumonisin B1 is reported to cause neurotoxic syndrome called equine leukoencephalomalacia (ELEM) in horse (Rheeder *et al* 2002). FB1 is hepato carcinogenic to rats and is associated with pulmonary edema in swine (Nelson *et al* 1993). Ingestion of moldy corn, infected by *F. verticilloides* is linked with high incidence of human liver cancer and esophageal cancer, in the regions of South Africa and China (Jones *et al* 2001). The international Agency for Research on Cancer (IARC) evaluated FB1, as possible carcinogen to humans (Group 2B) (Domijan *et al* 2005). Mycotoxigenic *Fusarium* spp on paddy has been reported under unfavorable conditions during harvesting and processing. However, *Fusarium* contamination occurred when conditions are conducive to infection during harvesting or storage. Paddy crop is susceptible to fungal infection and subsequent deterioration during storage. Detection of *Fusarium* spp is crucial to prevent toxins entering the food chain. Aghili *et al* (2010) reported the presence of fumonisins from unpolished rice. *Fusarium moniliforme,* being the source of fumonisins, detected in naturally contaminated rice samples from fields or during stored with *Fusarium* species.

2.13.4 Moniliformin

Moniliformin (MON) was found widely in food and feeds infected by strains of *Fusarium* species in *Gibberella fujikuroi* species complex such as *F. proliferatum*, *F. subglutinans*, *F. avenaceum*, and *F. tricinctum* (Logrieco *et al* 2002). Desjardins (2006) showed that MON production in the GFSC was widespread but not universal. Most notably, many strains of *F. proliferatum, F. subglutinans* and *F. thapsinum* produced high levels of MON, but *F. verticillioides* very rarely produced MON. Also stated by Leslie *et al* (1995) that *F. verticillioides* produce little or no MON. Moniliformin is a naturally occurring sodium or

potassium salt of 3- hydroxycyclobut-3-ene-1, 2-dione, toxic to plant and animal species (Uhlig *et al* 2007). The mechanism of the acute toxicity of MON is believed to be the inhibition of pyruvate dehydrogenase, i.e., blockage of entry of pyruvate into Krebs's cycle and a decrease in mitochondrial respiration (Pirrung *et al* 1996). Moniliformin is suspected of causing the Keshan's disease, a human myocardial impairment reported in rural areas of China and South Africa (Bottalico 1998, Pineda-Valdes and Bullerman 2000).

2.13.5 Beauvericin

Beauvericin (BEA) is a mycotoxin produced by several *Fusarium* species, mainly by *F. proliferatum*, *F. semitectum,* and *F. subglutinans* (Moretti *et al* 1997, Logrieco *et al* 1998, Shephard *et al* 1999). Beauvericin was first isolated from the culture of the insect-pathogenic fungus *Beauverina bassiana* (Hamill *et al* 1969). The first *Fusarium* species identified as a beauvericin producer was an isolate of *Fusarium subglutinans* (Gupta *et al* 1991). Beauvericin is a bioactive cyclohexadepsipeptide containing an alternating sequence of three N-methyl L-phenylalanyl and three D-α-hydroxyisovaleryl residues. This toxin is a specific cholesterol acylotransferase inhibitor and it is toxic to several human cell lines by the induction of apoptosis and DNA fragmentation (Logrieco *et al* 2002). Beauvericin are produced both in pre-harvested and in stored products (Bottalico *et al* 1989, Logrieco *et al* 1995, Moretti *et al* 1997). Most of FUM-producing *Fusarium* species also produce BEA (Desjardins 2006). According to Leslie *et al* (2004), *F. konzum* produce variable but generally low to moderate amounts of BEA (4 - 320 µg/g). Many strains of *F. verticillioides* (67%) and *F. proliferatum* (50%) did not produce BEA while the others produced BEA at low levels. One strain of *F. subglutinans* produced BEA but it did not produce FUMB1. Two strains of *F. konzum* produced both MON and BEA at low levels but only one strain produced FUMB1 at a low level (Darnetty and Salleh 2013). *F. succisae* have been shown to produce beauvericin (Santini *et al* 2012).

2.13.6 Fusarin C

It is one of a class of acyl-tetramic acids which are found in slime moulds, terrestrial fungi, marine fungi and marine sponges. The class includes compounds such as fuligorubin (3) from a slime mould (Steffan and Angew) zopfiellamide A (Nicholson *et al* 2001) from a marine fungus (Daferne *et al* 2002) and pramanicin (Leslie *et al* 1996) from a terrestrial. A homologous gene in *F. venenatum* and 11.9 kb fusA gene in *F. moniliforme* are responsible for the early stages of fusarin biosynthesis (Song *et al* 2004).

2.14 Role of Secondary metabolites in foot rot disease

Gibberellic acid (GA) has been elucidated as the real causative agents of "bakanae" disease (Yabuta *et al* 1937). Fusaric acid (5-butylpicolinic acid, FA) is a mycotoxin with low to moderate toxicity to animals and humans, but with high phytotoxic properties (Niehaus *et al* 2014). The role of GA_3 and FA in pathogenicity has been reported by many researchers.

This has been attributed to the fact that fusaric acid and gibberellic acid produced by *F. verticillioides* caused stunting and elongation, respectively in many species of plants besides rice (Sood 1964, Young-Ah *et al* 2013, Brown *et al* 2014). A positive correlation between GA_3 production in culture and foot rot has been observed (Sunder and Satyavir 1998b). Ma *et al* (2008) reported that *F. fujikuroi* usually produce large quantities of gibberellic acid (GA_3) in cultures and induced bakanae symptoms in experiments with artificial inoculation. They also found a significant correlation between the length of seedlings treated with GA_3 and bakanae infection. During recognition of the host, followed by pre-penetration morphogenesis, and/or pathogen establishment in the plants, gibberellic acids produced by the fungus accumulate within and around rice roots. High concentrations of GA resulted in hypertrophied cells in the parts of rice found above ground and caused abnormal internode elongation of the stem. As the disease progressed, infected plants become week and cannot support their weight due to lack of sturdiness and it topple over and die (Hwang *et al* 2013).

2.15 Bio-chemical variability

Gibberellic acid is a plant growth hormone that was first isolated from *G. fujikuroi* and fusaric acid is a polyketide-derived secondary metabolites produced by multiple species of the fungal genus *Fusarium*. *Fusarium* species within GFSC complex are able to produce mycotoxins that can affect human and animal health (Leslie *et al* 1992). Generally, *F. verticillioides* can produce fumonisins, *F. fujikuroi* can produce moniliformin, fumonisins and beauvericin, and *F. proliferatum* is able to synthesize fumonisins, fusaproliferin and beauvericin.

Kaur *et al* (2011) reported the presence of fusaric acid and gibberellic acid by thin layer chromatography and by calorimetric method from culture filtrate of *F. moniliforme*. They showed that all the isolates were positive for the production of GA but not all the isolates produced fusaric acid. The production of FA and GA differs according to the strain of the fungus that explained the variability in the production of elongation and stunting symptoms in the plant (Zainudin *et al* 2008a). Sunder and Satvir (1998a) reported that the culture filtrates of the fungus were shown to be able to induce bakanae symptoms in rice seedlings, but this phenomenon was not common among isolates of bakanae pathogens (Ou 1985), indicating variations in GA_3 production. Srivastava *et al* (2003) screened nineteen *Fusarium* strains to select potential strains for the production of gibberellin in the culture media in flasks and quantitative estimation was done spectrophotometrically which showed the presence of gibberellin in variable amounts. Bhalla (2010) analysed twenty eight strains of *Fusarium* for qualitative and quantitative estimation of gibberellins by thin layer chromatography and high performance liquid chromatographic (HPLC) method respectively and according to which categorized as low producer (100 mg), moderate producer (100-250 mg) and high producer (>250 mg). The biochemical background of GA biosynthesis by *F.*

moniliforme has been well characterized. Variability observed for gibberellin production by the different strains of *Fusarium moniliforme* may be due to the different metabolic pathways of gibberellin productions (Bhalla *et al* 2010). It was established that GA12-7-aldehyde is a branch point to the various GAs and several pathways have been established from this branch point (Bearder *et al* 1975, Tudzynski *et al* 1999). Differences mainly in the position and sequence of hydroxylation and more than one pathway from GA12-7-aldehyde may be the reason for variation in gibberellin production by different fungal strains. Other reason may be the differences in the geographical regions (Rojas *et al* 2001).

Fusaric acid (FA) production was shown to be highly variable within the strains of the same *Fusarium* spp. Production of FA could be used to separate *F. verticillioides* and *F. subglutinans* from other *Fusarium* species isolated from rice with bakanae symptoms. Recently, Niehaus *et al* (2014) identified and characterized the fusaric acid gene cluster in *F. fujikuroi* consisting of the PKS-encoding core gene and four co-regulated genes, FUB1–FUB5. They reported FUB1 and FUB4 were necessary for the biosynthesis of the secondary metabolites.

2.16 Genetic variability

Species identification of *Fusarium* based on morphological characteristics is complicated and time-consuming (Leslie and Summerell 2006). In addition, species of *Gibberella fujikuroi* species complex have similar morphologies and differentiation of *F. fujikuroi* from *F. proliferatum* morphologically is almost impossible. Therefore, molecular identification of fungal species by DNA sequence has been used to support morphological identification of *Fusarium* species. The differences in nucleotide sequences of genes have been used to support morphological identification of *Fusarium* species (Bashyal *et al* 2015). Phylogenetic analysis of DNA sequences has been used for differentiation and evaluation of the genetic relationship among closely related *Fusarium* species (Young-Ah *et al* 2013). A comparison at the DNA sequences level provides accurate classification of fungal species and elucidates the evolutionary, ecological relationships among diverse species (Mule *et al* 2005). Different molecular based techniques have been used to differentiate different populations of *F. moniliforme*. RAPD-PCR technique is a useful tool for differentiating between species and formae species of the genus *Fusarium* either alternatively or complementary to methods based upon morphological and pathological characteristics (Fadly *et al* 2008). Different techniques such as Random Amplified Polymorphism DNAs (RAPD) (Amoah *et al* 1995), Amplified Fragment Length Polymorphism (AFLPs) (Petrovic *et al* 2013) and CHEF gel karyotypes (Xu *et al* 1995) have also been used to differentiate the members of *G. fujikuroi* species complex. RAPD, AFLP and RFLP (Restriction Fragment Length Polymorphism) analysis have been used for molecular characterization of *F. verticillioides* (Moretti *et al* 2004, Patino *et al* 2004). Presently, *Fusarium* spp. were commonly characterized at species level by translation

elongation factor 1-α (TEF) gene sequencing (Pra 2010, Wulff *et al* 2010, Petrovic *et al* 2013), internally transcribed spacer regions in the ribosomal repeat region (ITS1 and ITS2) and β-tubulin (tub2) (O'Donnell *et al* 1998, Bashyal and Aggarwal 2013). Based on the analyses, it has been known that not all sequences work equally well for all species and translation elongation factor-1α gene (TEF1) gene [primer ef1 (5'- ATGGGTAAGGA (A? G) GACAAGA C-3') and primer ef2 (5'- GGA (G? A) GTACCAGT (G? C) ATCATGTT- 3')] being the most widely accepted across the genus because it is highly informative at the species level and non-orthologous copies of the gene have not been detected in the genus (Geiser *et al* 2004). However, the ITS regions do not work well within the *Liseola* section (Bashyal *et al* 2012). It is because many fusaria within the *Liseola section* possess non-orthologous copies of ITS2, which can lead to incorrect phylogenetic inference (O'Donnell *et al* 1998). The histone H3 (H3) and β-tubulin (BT) genes were also used for species identification of *Fusarium* (Young-Ah *et al* 2013). Recent developments in molecular systematics have revealed that the *Gibberella fujikuroi* complex includes at least 50 distinct species or phylogenetic lineages. Of these, 34 species have been formally described using morphological characters, 10 have also been described based on sexual fertility and at least 20 species produce one or more mycotoxins (Kvas *et al* 2009).

Multiple alignment of translation elongation factor (TEF) gene sequences of different *Fusarium* spp., showed a deletion of six nucleotides in *F. fujikuroi* sequence and a two nucleotide polymorphism in the same region of *F. proliferatum* sequence. These elements of variability were used to develop a conventional and real-time PCR assay for diagnosis. The species specific primer pairs (Fuji1F/TEF1R and Proli1F/TEF1R) gave a product of 179 and 188 bp for *F. fujikuroi* and *F. proliferatum,* respectively. Primer specificity was confirmed by analyzing the DNA of the most representative species of the GFSC and strains of *Fusarium* spp. isolated from rice plants and seeds. These specific primers were successfully used to detect fungal presence directly from infected rice tissues or seeds and providing a rapid tool for the early detection of pathogen contamination (Amatullia *et al* 2010). Quazi *et al* (2013) identified *F. proliferatum* based on molecular identification using species specific primer pairs and confirmed with sequencing. O'Donnell *et al* (1998) delineated *G. fujikuroi* complex into three lineages, designated as the African, Asian, and American clades.

Iram and Ahmed (2007) studied the polymorphism and genetic variations within the population of *Fusarium* spp. using RAPD and established correlation between taxonomical and genetical characters of fungi. Molecular identification of *Fusarium* spp. associated with bakanae disease of rice in Italy and their pathogenicity have been assessed by Amatullia *et al* (2010). They identified three clusters composing of *F.fujikuroi*, *F.proliferatum* and *F. verticilloides* and grouped under *Gibberella fujikuroi* complex on the basis of EF-1α, confirming that it is a good marker for species identification among *Fusarium* spp.

Damadzadeh (2003) determined the morphological and molecular characteristics of *F. verticillioides* using RAPD. Tripathi *et al* (2011) studied DNA polymorphism among 30 isolates of *F. verticillioides* isolated from seeds of 12 different crops. All isolates produced a monomorphic common band of 400 bp with the primer OPB1, which may be useful for developing strain specific SCAR marker for identification of *F. verticillioides* isolates. Development of PCR assays for an easy and sensitive identification of *Gibberella fujikuroi* anamorphs in maize kernels were described by Oller *et al* (1999). Genetic diversity among *F. verticillioides* species and isolates have been analysed by Huang *et al* (1997) using vegetative compatibility group (VCG) and from RAPD techniques and concluded that the cluster analysis of the RAPD data showed a very good agreement with the VCG grouping. Biochemical and molecular approaches have been used by Daroda *et al* (2001) to characterize and to monitor the activation of *F. moniliforme* endoPG during infection of maize and revealed an unknown variability in endoPG polypeptide structure among *F. moniliforme* strains. Thirty-two strains of *F. subglutinans*, six strains from *F. verticillioides* and five strains of *F. proliferatum* isolated from maize in Austria were studied by Youssuf *et al* (2002) using RAPD and results indicated that these *Fusaria* species were distinct species and hence RAPD markers can be quick and reliable for differentiating them. Bahmani *et al* (2012) assessed the genetic variability by RAPD analysis of 24 isolates of *F. verticillioides* in order to compare the overall similarity among these isolates at DNA level using seven OPA primers. Sreenivasa *et al* (2008) used one set of genus specific primers ITSF and ITSR to differentiate 103 *Fusarium* isolates from maize samples of closely related genes. Detection and identification of *Fusarium verticillioides* species was done by using a newly designed reverse primer VERT-R (5'- CGA CTC ACG GCC AGG AAA CC -3') based on an intergenic spacer sequence (IGS) combined with an already designed forward primer VERTF-1 (5'-GCG GGA ATT CAA AAG TGG CC -3'). Out of 103, 84 isolates of *F. verticillioides* were reported to score positive for the primers for confirmation of fumonisin production. Based on genome information of *F. verticillioides*, 109 SSR markers were developed by Xu *et al* (2012) which showed high polymorphism and high diversity among *F. verticillioides* isolates from maize. Hsuan *et al* (2010) isolated *Fusarium* from rice, sugarcane and maize and identified as *F. verticillioides, F. sacchari*, *F. proliferatum F. subglutinans, F. fujikuroi* and *F. oxysporum* by restriction analysis of intergenic spacer (RFLP-IGS) using *Alu*I, *Eco*88I, *Rsa*I and *Xho*I. Based on RFLP-IGS analysis, variability was observed within and between species of *Fusarium* from rice, maize and sugarcane. Twenty-five haplotypes showed high levels of variations and the isolates were clustered using UPGMA cluster analysis and estimate the intraspecific and interspecific variability. On the basis of their study, they clustered the isolates of *F. fujikuroi* from rice together with isolates of *F. proliferatum* from rice and maize with a similarity value of 88–100% and similarity value ranging from 92–

100% of *F. verticilliodes* from maize and sugarcane were clustered together and two isolates from rice formed another cluster. *Fusarium* isolates were identified using the translation elongation factor 1-α (TEF) gene region. *Fusarium verticillioides* was the most prevalent species representing 69.6% of the isolates. PCR analysis using the species-specific primers VER1/2 and PRO1/2 confirmed 98.5% and 85.1% of the morphological identifications of *F. verticillioides* and *Fusarium proliferatum* respectively (Rahjoo *et al* 2008). Darvishnia (2013) grouped thirty isolates of *Fusarium* spp. into seven phylogenetic groups (clades) and isolates of *F. nygamai*, *F. pseudonygamai*, *F. proliferatum* var. *proliferatum*, *F. proliferatum* var. *minus* and *F. fujikuroi* isolates formed a monophyletic group and *F. solani*, *F. tricinctum*, and *F. sporotrichioides*, *F. pallidoroseum*, *F. semitectum* and *Fusarium* spp. constituted into another monophyletic groups. Restriction analysis of the IGS regions was used to characterize *Fusarium* species section *Liseola* and to discriminate closely related species as well as to clarify their taxonomic position (Heng *et al* 2012). Five *Fusarium* species isolated from rice, sugarcane and maize collected from various locations in Peninsular Malaysia were characterized based on restriction patterns generated by the six restriction enzymes, *Bsu*151, *Bsu*RI, *Eco*RI, *Hin*6I, *Hin*fI, and *Msp*I (Heng *et al* 2012). Young-Ah *et al* (2013) used TEF and H3 to identify the *Fusarium* species from the samples collected from 10 Asian countries. SSRs are considered the most robust and efficient markers in population genetics. Twenty-eight SSRs, all derived from expressed sequence tags (ESTs), were found and tested by PCR on a sub-sample of *F. fujikuroi* isolates (Aragona *et al* 2012). The products were detected by polyacrylamide gel electrophoresis (PAGE), 27 SSRs gave the expected amplicon but only 3 resulted polymorphic, with an average number of alleles per locus ranging from 2 to 5. A preliminary statistical analysis on the distribution of polymorphisms in 135 isolates belonging to 5 pathogen populations showed 8% of molecular variance among populations and 92% within populations. Taking into account the low number of polymorphic SSRs found in *F. fujikuroi* published sequences, and the need of a larger number of markers for population structure analysis has been indicated.

2.17 Evaluation of germplasm lines

Bakanae disease is an important fungal disease in the world and has been a major problem facing world-wide and host resistance is the effective strategy to control the disease. It is difficult to develop bakanae resistant rice varieties due to the high genetic variation of the causal pathogens (Serafica and Cruz 2009). Many pathologists have argued that breeding resistant rice is the best way to prevent and eliminate bakanae disease rather than using hot water or fungicides to kill the fungus. As the first step in breeding disease-resistant varieties, an accurate and large-scale screening method must be developed to identify the disease-resistant genes and analyze genetic diversity (Kim *et al* 2014).

Many different sources of inoculum have been used for artificial inoculation, cultured

fungal spores (Khokhar and Jaffrey 2002, Ma *et al* 2008, Zainudin *et al* 2008a, Park *et al* 2008, Iqbal *et al* 2011), infected soil (Saremi *et al* 2008), infected soil and oat mixtures (Rajagopalan 1961). Kim *et al* (2014) reported an inoculation method for microconidia of *Fusarium fujikuroi* using a tissue embedding cassette and seedling tray, a new technique for large scale screening rice accessions to evaluate resistance to bakanae which was fast and reproducible for accurately evaluating resistance disease in rice. Standards for evaluating the inoculated rice seedlings as healthy or unhealthy were also established. They evaluated 10 rice cultivars to identify the disease symptoms produced by *F.moniliforme* CF283 isolate. They found that symptoms were present at a spore concentration of $1X10^6$ spores/ml, sufficient for differentiating susceptible and resistant cultivars.

The protocol of inoculum free varietal screening method for bakanae disease of rice was developed to find out resistant varieties from the huge collections of germplasm bank (Hossain *et al* 2013). Several varietal screening methods were tested along with dipping dry seeds into millipore membrane filtrate of spore suspension of the pathogen and gibberellic acid (GA_3) in different concentrations. It was found that GA_3 can be used to screen out susceptible rice varieties against bakanae disease. The method which requires the use of GA_3 is easier than the other methods for mass screening as it does not require maintaining living culture of the pathogen. In a study at Northwest Iran, Saremi *et al* (2008) reported that Binam cultivar was resistant to the foot rot disease and has the highest yield and the disease was reported to be more prevalent in areas where susceptible varieties were being cultivated continuously. Ma *et al* (2008) screened 32 rice genotypes carrying different dwarf or semi-dwarf genes and the study revealed two dwarf genes *sd1* and *d1*. *sd1* was sensitive to GA_3 and susceptible to bakanae disease and *d1* was sensitive to bakanae but insensitive to GA_3. They also reported genotypes carrying *d29, sd6 or sdq (t)* genes showed resistance to bakanae. The resistivity was reported to vary according to the finness and coarseness of the cultivars in Basmati. The fine cultivars were susceptible to foot rot disease as compared to the coarse cultivars (Khokhar and Jaffrey 2002, Ghazanfar *et al* 2013). Iqbal *et al* (2011) reported two varieties (IR-6 and KSK-133) as resistant, three varieties (Bas-Pak/kernel, Bas-198 and Bas-370) as moderately resistant, two varieties (Bas-385 and Bas-Super) as susceptible in Pakistan. Three coarse varieties i.e. IR-6, KSK-133, KS-282 and six fine rice varieties i.e. Basmati-198, Basmati-2000, Basmati Pak, Basmati-Super, Basmati-370 and Basmati-385 were screened for the resistance against foot rot disease (Ghazanfar *et al* 2013). The screening results revealed that IR-6 and KS-133 were found as resistant with 18.80 and 19.82 percent plant infection, while varieties Basmati-385 and Basmati-Super with 61.99 and 61.04 percent plant infection exhibited susceptible reaction. Their findings indicated that coarse varieties were more resistant to bakanae disease as compared to fine ones. Bagga and Kumar (2000) reported thirteen highly resistant lines and seven moderately resistant lines against *Fusarium*

moniliforme out of the eighty-five genotypes evaluated in an experiment. Pannu *et al* (2013) reported certain genotypes of rice and basmati rice showing resistance against foot rot under the field condition.

CHAPTER-III

MATERIALS AND METHODS

3.1 Survey and collection of diseased samples

The foot rot infected plant samples were collected from different districts of Punjab during *Kharif* season (July-August). The samples were put in air tight container, dried and kept in refrigerator. Later, the diseased portion of the samples were cut and trimmed for further use for isolation and purification of the pathogen.

3.2 Isolation, purification and maintenance

A total of 38 isolates of *Fusarium moniliforme* were collected from the foot rot infected fields of different agro-ecological zones of Punjab. The diseased samples were cut into 3-4 mm size pieces and were surface sterilized with mercuric chloride (0.1%). The cut pieces were then placed equidistantly on Potato dextrose agar (PDA) media in each Petri plate under complete sterile and aseptic conditions. Plates were then incubated at 25±2°C in a BOD incubator. The cultures obtained were purified and examined microscopically to identify the fungus associated. Different isolates of *Fusarium* spp. were purified by single spore culture method and multiplied on PDA for further studies. These isolates were labeled as FR1-FR38 (Table 1). The isolated and purified cultures were preserved and labeled in slants. The isolates were identified morphologically using 'The *Fusarium* Laboratory Manual' (Leslie and Summerell 2006) and also confirmed the species as *F. verticilloides* (*F. moniliforme*) with the help of species specific primers VERT1 and VERT2.

3.3 Cultural and Morphological Characterization

3.3.1 Characterization of Cultural features and growth parameter

The growth characters and morphological characteristics of *F. moniliforme* were studied on three different solid media *viz*., Potato Dextrose Agar (PDA), Soil Extract Agar (SEA) and Spezieller Nahrstoffarmer Agar (SNA). The composition of different media is given below in Table 2. The isolates were cultured and multiplied on these three media in Petri dishes for comparisons of cultural characteristics. The three media were prepared and sterilized in the autoclave at 15 *psi* for 30 minutes. Each medium of 20 ml was poured into Petri dishes of 85mm diameter. The mycelium discs of 5mm from actively growing culture of each isolate were cut with the help of a cork-borer and inoculated in the centre of the Petri dishes and incubated at 25±2°C. Each treatment was replicated thrice. Fungal growth rate and pigmentation, radial growth, colony texture, surface nature, zonations, exudations, production of sporodochia and production of chlamydospores in all the isolates were recorded on all the three media. Colony diameter and growth rate were observed on alternate day upto 8th day and other colony characters were observed after 15th day. Average of the colony diameter and growth rate for three replicates of each isolate was calculated.

Table1. Isolates of *Fusarium moniliforme* collected from different locations of Punjab

Isolates ID	Location (District)	Isolates ID	Location (District)
FR1	Moonak (Sangrur)	FR20	Ludhiana (Ludhiana)
FR2	Moonak (Sangrur)	FR21	Bhausand (Sangrur)
FR3	Kamo Majra Kalan (Sangrur)	FR22	Nawagaon (Sangrur)
FR4	Shaheed Bhagat singh Nagar (Shaheed Bhagat singh Nagar)	FR23	Dhuri (Sangrur)
FR5	Shaheed Bhagat singh Nagar (Shaheed Bhagat singh Nagar)	FR24	Kalanaur (Gudaspur)
FR6	Gurdaspur (Gurdaspur)	FR25	Pathankot (Pathankot)
FR7	Ludhiana (Ludhiana)	FR 26	Muktsar Sahib (Muktsar Sahib)
FR8	Ludhiana (Ludhiana)	FR 27	Sahiwal (Gurdaspur)
FR9	Dina Nagar (Gurdaspur)	FR 28	Shaheed Bhagat Singh Nagar (Shaheed Bhagat singh Nagar)
FR10	Dorangla (Gurdaspur)	FR 29	Ludhiana (Ludhiana)
FR11	Dhariwal (Gurdaspur)	FR 30	Bahadurpur (Ludhiana)
FR12	Dhariwal (Gurdaspur)	FR 31	Research farm PAU (Ludhiana)
FR13	Gurdaspur (Gurdaspur)	FR 32	Rasulpur (Ludhiana)
FR14	Gurdaspur (Gurdaspur)	FR 33	Rasulpur Bet (Gurdaspur)
FR15	Gurdaspur (Gurdaspur)	FR 34	Rasulpur Bet (Gurdaspur)
FR16	Jhakhaladi (Pathankot)	FR 35	Kala Nangal (Gurdaspur)
FR17	Muktsar Sahib (Muktsar Sahib)	FR 36	Regional Station (Gurdaspur)
FR18	Amritsar (Amritsar)	FR 37	Nurmahal KVK (Jalandhar)
FR19	Research farm PAU (Ludhiana)	FR 38	Badshahpur (Kapurthala)

3.3.2 Characterization of conidial structures

Spezieller Nahrstoffarmer Agar (SNA) medium was used for examining micro-conidia and the formation of chlamydospores and Carnation Leaf Agar medium (CLA) was used for examining the macro-conidial structures and sporodochia. Cultures were also grown on water agar supplemented with KCl for observation of the production of micro-conidial chains. Compositions of CLA and water Agar are given in table 2.

Table 2: Composition of different culture media

Media	Components	Quantity
1. Potato dextrose Agar	Dextrose	20g/L
	Agar	20g/L
	Potato	250g/L
	Water	1000ml
2. Spezieller Nahrstoffarmer Agar	K_2HPO_4	1g
	KNO_3	1g
	$MgSO_4.7H_2O$	0.5g
	KCl	0.5g
	Glucose	0.2g
	Sucrose	0.2g
	Agar	20g
3. Water Agar infused with KCl	KCl Agar	4-8g/L
	Water Agar (2%)	20g agar/ L of water
4. Carnation Leaf Agar medium	Water Agar (2%)	20g agar / L of water
	Sterile carnation leaf pieces	3-5mm^2/ 2ml of medium

Special treatment for sterilization purpose was given for Carnation Leaf Agar medium (CLA) medium. Fresh carnation leaves were cut into 5-8 mm^2 pieces and oven dried for 2hrs until brittle. The oven dried sterile leaves were packed in aluminum foil and sterilized by gamma radiation (2.5 mega rads). The sterile 5-6 leaves were put on the plates containing Water Agar (WA) media. The *Fusarium* isolates were then inoculated on these plates.

The isolates were grown on different media for 15 days at 25±2°C. Slides were prepared from 15 days old culture and observed under microscope Leica DM 3000 to observe the production of micro-conidia and macro-conidia. The size of micro-conidia and macro-conidia were measured for each isolate separately at 40X using image analyzer software. For every isolates, size of 50 spores per microscopic field was measured and average was worked

out. All the microscopic observations, measurements of the spores and photographs were carried out using Leica DM 3000 microscope. Further identification was performed using 'The *Fusarium* laboratory Manual' by Leslie and Summerell (2006).

3.4 Pathogenicity of representative isolates on susceptible cultivar Pusa1121

The isolates FR1-FR38 were tested for their pathogenicity under green house conditions in sterilized soil as described by Wulff *et al* 2010 and Bashyal *et al* 2015. The soil was sterilized twice in autoclave at 15 *psi* for 30 minutes. The sterilized soil was then filled in trays. The inoculated seeds of susceptible cv. Pusa1121 were used for the test. The seeds were artificially inoculated by dipping in the conidial suspension of each isolate of *F. moniliforme* separately. For preparing the conidial suspension, all the *Fusarium* isolates were grown on PDA in Petri plates at 27±2°C for 10 days. The fully grown cultures were flooded with sterilized distilled water and scraped with a sterile spatula. The resulting suspension was filtered through double layered muslin cloth and final concentration of the suspension was adjusted to $4x10^4$ conidia/ml using haemocytometer. The seeds were then soaked in the suspension for 3 hrs at room temperature. The inoculated seeds were then sown in the trays containing sterilized soil. Each treatment was replicated thrice. Observations were recorded after 15-20 days of sowing in terms of percent disease incidence (D.I.). The number of foot rot infected elongated and stunted plants were recorded. The *Fusarium* isolates were then, categorized as moderately virulent (2-25%), virulent (26-50%) and highly virulent (51-100%) on the basis of pathogenicity following the standard evaluation system of the International Rice Research Institute (Anonymous 1996).

3.5 Characterization of bio-chemical variability of different isolates of *Fusarium moniliforme*

3.5.1 Qualitative and quantitative analysis of Gibberellic acid production

For estimation of gibberellic acid production, all the isolates were grown in 100ml of sterilized Czapek-Dox medium in three replicates for 12 days at 25±2°C. Each flask was inoculated with 5mm mycelial disc of individual isolate. After 12 days, mycelial mat was separated using Whatman filter paper no.1 and pH of the filtered product was adjusted to 2.5 by using 1N HCl (Hasan 2002, Bhalla *et al* 2009, Muddapur *et al* 2015). The filtered product was extracted with equal volume of ethyl acetate. The ethyl acetate layer was dried over Na_2SO_4 and evaporated in rotary evaporator. The suspended residue was dissolved in acetone and spotted on a silica gel TLC plate along with standard GA_3 (Sigma) and developed by using isopropanol: ammonia: water (10:1:1, v/v/v). The plates were sprayed with the reagent (3% H_2SO_4 in methanol and 50 mg $FeCl_3$). After spraying, the plates were heated in oven at 80°C for 10 min and observed under UV light.

Quantitative determination of GA was done by spectrophotometric method (Paleg 1965, Zainudin *et al* 2008b). To 25 ml of filtrate in a test tube, 2ml of zinc acetate was added

followed by 2 ml potassium ferrocyanide after an interval of 2 mins and centrifuged at 1000 rpm for 15 mins. Equal volumes of supernatant and 30% HCl were incubated at 20°C for 75 min. The blank sample was treated with 5% HCl and the absorbance of the test sample and blank was measured at 254 nm in spectrophotometer. The amount of GA present in the extract was calculated from the standard curve prepared using graded concentration of GA (1 mg in 10 ml- stock solution) and expressed in terms of µg/ml of the media.

3.5.2 Qualitative and quantitative analysis of Fusaric acid

For extraction of fusaric acid, the cultures were grown in 100 ml of sterile Czapek-Dox medium in three replicates for 15 days at 25±2°C.The mycelial mat were separated using Whatman filter paper no.1 after 15 days of incubation and the filtrate were retained and FA was extracted from it following the method of Pritesh *et al* (2010) with slight modifications. The pH of filtrate was adjusted to 3.5 – 4.0 with 2N HCl and resuspended in equal amount of ethyl acetate (100ml). It was then shaken well in a separatory funnel and left undisturbed for ½ an hr for separation of two layers. Upper layer of ethyl acetate was collected in a conical flask. Similarly, the extraction process was repeated thrice with the lower broth layer. All the extracts were pooled together. Ethyl acetate was then evaporated on a rotary evaporator at temperature 27°C. The residue was collected and suspended in 5ml ethyl alcohol and stored in a freezer until the sample is run on TLC plate. The plates were developed in n-butanol, acetic acid, ethyl acetate and distilled water (3: 2: 2: 2, v/v) solvent put in a glass chamber. Before spotting, the TLC plates were activated by keeping in the oven for 10 minutes. The activated plate was then spotted with the suspended residue of 30µl along with FA standard (Sigma) and run along with the solvent present in glass chamber. After one and half hours, the TLC plates were removed from the chamber and again dried in the air. It was then observed under long-wave UV light (365 nm) (Burmeister *et al* 1985, Pritesh *et al* 2010). Those isolates that produced fusaric acid showed pinkish bands on the plates. This same extract was then used for quantitative estimation of FA. The bands that observed on TLC plates were scraped with a scapel and eluted in 5ml ethanol and kept undisturbed for 20 mins. When the silica material settled down, the extracted FA were eluted in the ethanol, it was then observed under spectrophotometer at 254nm and the absorbance of different samples were recorded. The amount of FA present in the extract was calculated from the standard curve prepared using graded concentration of FA (1 mg in 10 ml-stock solution) and expressed in terms of µg/ml of the media.

3.6 Identification of species using species specific primers and study of variability using Simple Sequence Repeats (SSR) primers

3.6.1 Culture preparation

For extraction of the fungal DNA, the pure cultures of the fungus was grown on 100ml Potato dextrose broth (PDB) medium in 250ml flasks for 10 days at 25±2°C. After10

days of incubation, the fungal mycelial mat was obtained by filtering with Whatman paper No.1.

3.6.2 DNA extraction

Genomic DNA was extracted from 38 pure cultures of *Fusarium moniliforme* by using mini-prep Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompon 1980).

1. The fungal mat was crushed to form a fine powder in liquid nitrogen using sterilized pestle and mortar. The powder so obtained was collected in 2ml centrifuge tubes.
2. CTAB buffer of 800 µl (Table 3) containing 1% mercaptoethanol was added to the powder and the tubes were incubated in water bath at 65°C for 1 hour. Contents of the tubes were mixed gently after every 15 minutes by inverting the tubes.

Table 3: Composition of Cetyl Trimethyl Ammonium Bromide (CTAB)

Components	Stock concentration	Final Concentration
1 M Tris-HCl, pH8.0	20.0 ml	100 mM
5M NaCl	28.0ml	1.4 mM
0.5M EDTA	4.0M	20mM
10% CTAB	20.0 ml	2.0%
Sodium bisulphate	0.5 g	0.5%
Mercaptoethanol	1.0 g	1.0%
Double distilled H_2O	28.0 ml	-

3. After incubation, 800 µl of chloroform: Isoamyl alcohol (24:1) was added. The samples were cooled down to room temperature and for proper mixing the tubes were put on shaker at 70 rpm for 30-45 min. After shaking, the samples were centrifuged at 10,000 rpm for 15 min in a micro-centrifuge.
4. The supernatant was collected from the tissue debris in fresh 1.5 ml/centrifuge tube using pipette and RNase treatment was given by adding 10 µl of RNase to each tube followed by incubation at 37°C for 30 min.
5. Then 800 µl of chilled isopropyl alcohol was added followed by gentle inversions. After this tubes were incubated at 4°C for 15 min.
6. The tubes were again centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and pellet was washed with 70% ethanol. The pellet was then air dried and dissolved in 50 ml of Trisaminomethane-Ethylenediaminetetraacetic Acid (Tris EDTA) buffer (Table 4). DNA of all the isolates was stored at -20°C till further use.

Table 4: Composition of buffer Trisaminomethane-Ethylenediaminetetraacetic Acid (Tris EDTA) buffer

Components	Final Concentration	Volume per 100 ml
1 M Tris-HCl	10 mM	1.0 ml
0.5M EDTA	1 mM	0.2 ml
Double distilled H_2O	-	To make volume upto 100 ml

3.6.3 Estimation of quantity and quality of genomic DNA

Quantification of nucleic acids was performed by using NanoDrop™ 1000 spectrophotometer (ThermoScientific, Wilmington, USA). The optical surface of the spectrophotometer (Thermo Scientific Nano Drop™ 1000) system was cleaned by putting 1-2µl deionised water on the lower optical surface. The lever arm was closed and tapped few times to clean the upper optical surface and both the optical surfaces were wiped with tissue paper by lifting the lever. The Nano Drop software was clicked to open and “Nucleic acid” module was selected. Spectrophotometer was initialized by placing 2µl clean water onto the lower optic surface and selecting “Initialize” in the Nano Drop software. A “Blank” (Water and TE buffer in which no DNA sample was dissolved) measurement was performed by loading 2µl of TE buffer. Measurement of the nucleic acid sample was done by loading 2µl of sample and selecting “Measure”. Pure nucleic acid typically yield a 260/280 ratio of ~1.80 for DNA. Value less than 1.8 indicates presence of significant concentrations of proteins and a ratio more than 1.8 indicates presence of RNA.

3.6.4 Dilution of Original genomic DNA

The concentration of fungal DNA was diluted to adjust the concentration to 25ng/µl for use in polymerase chain reactions.

3.6.5 Selection of primers

The primer sequences were got synthesized from Promega as 25 nmol in dry form. These primers were diluted to 10µM working concentration using double distilled water. For the present study, two set of primers specific for *F. verticilloides* and *F. proliferatum* were used (Table 5) for identification of the *Fusarium* species.

Table 5: Species specific primers used for identification of *Fusarium* species

Primer name	Sequence	Product size (bp)	Reference
VERT 1 VERT 2	GTCAGAAATCCATGCCAGAACG CACCCGCAGCAATCCATCAG Specific for *F. verticilloides*	800 bp	Patino *et al* (2004)
PRO 1 PRO 2	CCTTCCGCCAAGTTTCTTT TGTCAGTAACTCGACGTTGTTG Specific for *F. proliferatum*	585 bp	Mule *et al* (2004)

Fifty SSR markers were designed for diversity studies. The primers are listed in Table 6 as given by Xu *et al* (2012) and 27 primers which gave amplification were selected.

3.6.6 Polymerase chain reaction analysis

3.6.6.1 Dilution of primers

Each primer was dissolved in 100µl of 1X Tris EDTA buffer (Table 4) and diluted further with deionized water to the working concentration of 10µM. The primers were diluted as per following formula:

$$\mu M \text{ of oligo in } 100\mu l \text{ of solution} = \frac{OD}{10}$$

Table 6: Simple Sequence Repeats (SSR) primers used to study diversity

Sl.no.	Primer	Repeat motif	Sequences	Expected size (bp)	Annealing temperature (^{0}C)
1	1H06	(GGA)14	GAGCCCCGAGGAGAACCAT CTTGCCGTCTTCAGCATC	321	51
2	1H09	(ACAG)6	TCCACCAAACGAACAGT TTGCCGTAAAGGAGACAT	197	47
3	2H13*	(GGA)41	TATCTGCCCATTGTGCTT CCTGGTATCGCTTTTGTAG	275	48
4	2H17*	(TCTG)35	GAGTTGGAGCCTGGATGT GTAGTGACGATTGGACGC	371	51
5	3H14	(ACCT)7	GCAAGTCTCGCCGTGTAA GCTGAAGATAGTGACGATGGA	272	52
6	3H19*	(GA)13(AG)7	ACCTTTGTCAGCCACTCCA CTCTGCTTCTGTGCCTACCT	188	57
7	4H15	$(TACA)_7$	TGAACCAGGCACAGAAAGC AATCGGTGGACGAAGGGA	203	50
8	4H16	$(TACA)_8$	CGGAAACGACAGACCAAA AACTCGCTCGCTCCAAAG	221	45
9	5H07	$(GAAA)_{16}$	GTAGCGGTTATGGTTCCCTC CGTGATGCGATTCTGGTTG	363	55
10	5H09*	$(TGTT)_7$	TGGCGAGATAAATGAACG AAAGCCGCAGACAAAGAC	152	48
11	6H03	$(CAGA)_{14}$	GGCGTAGCTTAGCGAAAT AGACATGACCAGGATACAACT	332	53
12	6H05	$(GGT)_9$ $(ATG)_1$	GGGAGATGGAAAGGAAGTGT CTCGTCTATGCTCTGCTCGT	180	53
13	7H16*	$(AC)_{15}$	TACTCAGCCACCAGGACA TGGAAAGGAACAGAATAGGA	291	51
14	7H19	$(AC)_{13}$	GAAGACCACAGCCCATAAAGT ACAAGTGACCCGCCCATT	213	55
15	8H01*	$(AAG)_9$	TGGTATGATGGCTGGTTCG ACTAGAGGCTGGGTTGGTGT	485	57
16	8H02	$(TTC)_{13}$	ACCTACAAATCTCAGGAACCAA	465	58

			GCGAACAATAGCATCCAAAT		
17	9H05*	$(ATGG)_{11}$	AGGCACGAGTGGATAAGGC TTGGAAGCAAGCGAAGGA	221	51
18	9H10	$(CCAT)_{6}$	CTCGTGCTTCTGCTCCTT TGTGCCCAATACAATACCC	262	51
19	10H01*	$(ACA)_{11}$	ACCGCTCATCAGCACATCA GCCAAAGTCAAACCCAGTCA	218	51
20	10H09*	$(AGA)_{6}$	GAAGGCTACATTGACGACG AGACTGATGCGGGAGGAT	118	53
21	11H03*	$(GA)_{6}$	CGCTGGTCTTCTGTATTCTGC TGTATGATGTTGAGGCTTGTGA	409	59
22	11H07	$(TC)_{6}$	ATTCCTTCGCCCGTGCTT CGGTGGTCTTGTTGGGTTTG	353	57
23	1H01*	(CAT)9	CTGTTCGCTTACGGGTT CGAGGATGTCACGCTTAT	205	49
24	1H02*	(CCT)11	GGCAGAGGCAAATCAATC AGCCCAAGCTGTTATGGA	305	49
25	1H15	(CACT)5	TCAACTCGTTCCGTCCAG TCACCCAGTCAGAGCAAAA	340	50
26	1H16	(CT)10	ATCAGCAAACGATGAACGC GGGAAAGGGACGGAAAGT	433	51
27	2H05*	(GAT)10	TGCTGTGGGAATCTGAAC CGACGAAAACTGGACTGA	277	50
28	2H06*	(ATG)8	TAATGAGACGGCTGACGG GCACCAACTGCTGAGAAA	273	50
29	2H14*	(GTT)10	GGTTCCGCATTCTCAACA CTCGCAACGGCTCTTACA	400	51
30	2H15*	(GTT)24	GGAGGAGGTTTGATGGTT GAGCGTGTCACAGCAAGT	243	50
31	3H01*	(AGA)10	CCTTGAAGAACATGACGACG GCTCCCATAACAGCCACTAA	354	55
32	3H02*	(ATG)11	ATCACCAAAACAGAGCAAAG GTGGCTGAGAAAGACAAGAA	181	50
33	3H07	(ACCTT)11	AATGGGCATCGGTTCTGG GGGTTCGGGTTCCTCTTTTA	330	51
34	3H09*	(TGTA)9	ATGACGCCAGAAGAACGA CCATTGAGATGCAAAGGTG	348	51
35	4H01	$(AGCA)_{9}$	TACAGTGCGTGGATGGAA TGTGGTGGGAAGTGAGAC	421	50
36	4H09	$(AG)_{9}$ $(AT)_{10}$	TGCCCTGAGAACTCCATA ACGATAACACCAAACCAAG	302	49
37	5H04*	$(CTT)_{9}$	AGACTTCCTGGCTCTATCGT GCTGTATCCTGACTTGTTTCC	433	56
38	5H06*	$(GTCA)_{6}$	ACCCGACAGGAGTGGTAAGA GCAACGACCGAGACAGAAAT	434	56
39	5H10*	$(GTAT)_{7}$	ATGGGCTTGGCATTCTATC TTTCTTGTTGAGTCGTGGGT	409	52.5

40	5H12	$(GAAA)_7$	GGCACCAACATTCCTGACG AACCGCCTACAAGCACCAC	404	55
41	6H01	$(AGAC)_9$	AAAGGGCTGCCAGGAAAT AAAGTCAGAACGAAGACGGAGA	419	54
42	6H02	$(GTTC)_{14}$	AACGGTATAGCGAGAAACG GGACGGAGTCGAATGAAGT	222	52
43	7H05*	$(CGA)_{11}$	TTAGTAAGGGCGAAGAGG AACCAGAATGTTGAGGAGAA	487	50
44	7H06	$(ATGT)_9$	TACCTGAGACAGACAGATGC TTGGTGCTCCTATGTGCTA	345	53
45	10H05*	$(AG)_8$	AATAACCGAACAGACGAGTGAC GCCTGCTCTACAGCCCATAA	434	58
46	11H01*	$(AG)_{12}$	CGCAGACACGATTGAGAA GTTTGTGCCAGTGCCTTA	358	49
47	10H02	$(CATT)_7$	GCATCTGGCACCGTTGAA CAGCCTTATCCTTGACGACAT	294	54
48	10H03	$(ATT)_7$	GCGGGTATGACAGACAAACAG GCCAGACCAGCCAGCCAGACTTA T	293	65
49	10H07*	$(CCA)_6$	GGCAGCAAGATTCGGGATT GGAGATGCGAGTGAAGAAGGTA	484	57
50	11H06	$(TA)_7$	TATCCCAAGGCGACTGTTAC GTTGCTTATTAGAGGGCCTGAA	230	57.5

*The primers that showed amplification

3.6.6.2 Polymerase chain reaction mixture

Polymerase Chain Reaction (PCR) was performed in an Eppendorf Master cycler to study the polymorphism. PCR analysis was carried out for 27 SSR primers. It was carried out in the reaction volume of 30µl containing the 2µl template genomic DNA, 2.5 mM of 25 mM $MgCl_2$, forward and reverse primers, 200µM µl of 25mM dNTPs, 6 µl of 5X PCR buffer and 0.3µl of *Taq* polymerase (Promega) (5U/ µl). PCR profile and reaction mixture is given in Table 7 and Table 8 respectively.

3.6.6.3 Visualization of amplified product

The amplified products were visualized on 0.8 % agarose gel electrophoresis. For the purpose, 0.8 % agarose gel was prepared by dissolving agarose powder in 0.5X Tris Borate EDTA (TBE) buffer. The mixture was heated till the solution became transparent and clear. It was cooled down to 60°C with constant stirring. After cooling, 10µl Ethidium Bromide (10mg/ ml) was added to 135ml molten agarose. The agarose solution was poured into gel mould with combs. The combs were removed gently and the gel was placed in the gel tank for electrophoresis. PCR products (12µl) were loaded into wells with micropipette, along with the standard 100bp DNA ladder (Promega). After loading, the gel was subjected to electrophoresis at constant voltage of 5V/ cm for 1 hour. The gel was visualized under trans-illuminator and

Table 7: Polymerase chain reaction mixture for running Simple Sequence Repeats primers

Components	Stock concentration	Final concentration	Volume used
Template DNA	250-560 ng/ µl	30ng/ µl	2 µl
PCR colored buffered	5X	1X	6 µl
$MgCl_2$	25mM	2.5 mM	3 µl
dNTP mix	10mM	200µM	0.6 µl
Forward Primer	10mM	10 µM	1.5 µl
Reverse Primer	10mM	10 µM	1.5 µl
Taq polymerase	5U/ µl	1U	0.3 µl
Nuclease free water	----	----	15.1 µl
Total volume			30 µl

Table 8: Temperature profile used in PCR for SSR primers

Step no.	Cycling conditions	Temperature	Time
I	Initial Denaturation	94ºC	4 min
II	Denaturation	94 ºC	1min
III	Annealing	48-65 ºC	1 min
IV	Extension	72 ºC	1 min
V	Go to step II		35 cycles
VI	Final extension	72 ºC	7 min
VII	Store	4 ºC	

recorded with gel documentation system (Alpha Imager HP, USA). A permanent standard marker 100bp producing fragments between 100bp to 3000bp was loaded with sample. After loading, the gel was subjected to electrophoresis at constant voltage of 5 V/cm for about 1 hour. After electrophoresis, the gel was visualized under UV trans-illuminator and photographed using Alpha Innotech Multi Imager gel documentation system software programme from Alpha Innotech, California, USA.

3.6.6.4 Preparation of Tris Borate EDTA (10X stock)

For preparing Tris Borate EDTA (TBE) buffer, 108.0 g of Tris base, 53.30 g of Boric acid was dissolved in 600.0 ml of distilled water and 40.0 ml of EDTA (pH 8.0) were added (Table 9). All the constituents were dissolved and pH was adjusted to 8.0. The final volume was made up to 1000ml, sterilized by autoclaving and stored at room temperature.

Table 9: Composition of Tris Borate EDTA buffer (10X stock)

Component	Volume
Tris base	108.0 g
Boric acid	53.3 g
EDTA	40.0 ml
Distilled water	1000.0 ml

3.6.6.5 Scoring of SSR alleles

The SSR allele sizes were determined by the position of bands relative to the DNA ladder. Total number of alleles was recorded for each SSR primers in all the isolates under study by giving the number to amplified alleles as 0 for absence and 1 for presence of allele. The amplicon size was made out from 100 bp ladder run along with the PCR products on the gel. Polymorphic information content (PIC) that provides an estimate of the discriminatory power of a locus or loci, by taking into account not only the number of alleles that are expressed, but also relative frequencies of those alleles, was estimated using the following equation of Botstein *et al* (1980).

$$PIC = 1- n \Sigma (P_{ij})^2$$

where, P_{ij} is the frequency of jth allele in i^{th} primer and summation extendes over 'n' pattern.

3.6.6.6 Statistical analysis

The genetic diversity among the germplasm lines was carried out by computer software programme – DARwin 6.0 (Perrier and Jacquemond-Collet 2006). Dissimilarity matrix for SSR primers was constructed using Dice coefficient of associations to find out genetic relationships. The data were subjected to unweighted pair groups method with arithmetic mean (UPGMA) analysis to generate dendrogram. Data from 27 primers were used to estimate the dissimilarity based on Jaccard coefficient.

$$d_{ij} = b+c/a+(b+c)$$

where,

d_{ij} : dissimilarity between units i^{th} and j^{th}

xi^{th} and xj^{th} : variable values for units i^{th} and j^{th}

a : number of variables where xi^{th} = presence and xj^{th} = presence

b : number of variables where xi^{th} = presence and xj^{th} = absence

c : number of variables where xi^{th} = absence and xj^{th}= presence

where 'a' represents matched fragments, b and c are unmatched fragments. The 2a+(b+c) are the total number of fragments amplified in a particular set.

3.7 Survival of *Fusarium moniliforme* in soil

To investigate the soil borne nature of *Fusarium moniliforme*, an experiment was conducted under field conditions by incorporating infected straw in soil. There were eight treatments (Table 10). Eight plots were prepared. Four of the plots were artificially infested by adding straw infected with foot rot in the field and four of them were left uninfested. For artificial infestation of the field, infected plant debris was collected in October, 2013 after harvesting bakanae infected basmati cultivar Pusa 1121. The debris was chopped into small pieces of size 1cm and

Table 10: Survivability of *Fusarium moniliforme* in soil

Treatment No.	Treatments
T_1	Healthy seeds sown in infested soil
T_2	Healthy seeds sown in uninfested soil
T_3	Artificially inoculated diseased seeds sown in infested soil
T_4	Artificially inoculated diseased seeds sown in uninfested soil
T_5	Naturally infected seeds sown in infested soil
T_6	Naturally infected seeds sown in uninfested soil
T_7	Bavistin treated inoculated seeds sown in infested soil
T_8	Bavistin treated inoculated seeds sown in uninfested soil
T_9	Artificially inoculated infected seeds of susceptible cultivar Pusa1121 in un-infested soil

dried under shade. Chopped plant debris was then added and mixed in four plots of 2x2 m^2 in the month of November 2013 and were ploughed. The wheat crop was sown previously in all the plots. After harvesting wheat, the nursery of basmati cultivar Pusa 1121 was then sown in these plots in July, 2014 by giving different treatments

In T_1 and T_2, healthy seeds were sown in infested and uninfested soil respectively. For T_3, T_4, T_7, andT_8, seeds were inoculated by dipping for 3hrs in the culture suspension of *F. moniliforme* to ensure that all the seeds were infected. For T_7 and T_8, seed were then treated with Bavistin @ 2g/L for 12hrs before sowing. In T_9, infected seeds of susceptible cultivar Pusa1121 were artificially inoculated and sown in uninfested soil and this served as control. Observations on disease symptoms were recorded and the infected seedlings were counted.

3.7.1 Preparation of conidial suspension from culture of *Fusarium moniliforme* for artificial inoculation

Conidial suspension was prepared from 15 days old culture of *Fusarium moniliforme* multiplied on PDA by mixing in sterilized distilled water and shaken vigorously. It was then filtered through double layer of muslin cloth. The concentration of conidial suspension was adjusted to $4x10^4$ conidia/ml with the help of haemocytometer. The seeds were then soaked in the inoculum for 3hrs before sowing.

3.8 Host Range

F. moniliforme is a cosmopolitan fungus, having wide host range. There is a possibility that the major weeds in the rice field may serve as hosts. Therefore, nine different weeds found in the rice fields were tested for their susceptibility to *Fusarium moniliforme* during crop season 2014 under artificial inoculation conditions (Table 11). The weeds were uprooted at seedling stage from the rice fields and their roots were dipped for 3hrs in spore suspension of *Fusarium moniliforme* having concentration of $4x10^4$ spore/ ml and transplanted in the field. The plants were then observed for the development of the symptoms.

Table 11: Different weeds evaluated for studying host range of *Fusarium moniliforme*

Common Name	Scientific Name
Sanni	*Sphenoclea zeylanica*
Ammania	*Ammania baccifera*
Ghuein	*Fimbristylis tenera*
Madhana	*Dactyloctenuim aegyptiacum*
Joyweed	*Alternanthra sessilis*
Chhatri wala motha	*Cyperus iria*
Narhi gha	*Paspulum distichum*
Hairsedge	*Bulbostylis barbata*
Takri gha	*Digitaria sanguinalis*

3.9 Screening of germplasm Lines

Total of 134 germplasm lines of basmati (Table 12) were screened in field under artificial inoculation conditions. A pathogenic isolate of *F. moniliforme* was multiplied on PDA slants at 27-30°C for 15 days. The conidial suspension was prepared by harvesting conidia in sterilized distilled water by scrapping the cultures in the water and adjusted to a concentration of (4×10^4spores/ml) for inoculation.

3.9.1 Screening in transplanted rice

Thirty day old seedlings of all germplasm lines were uprooted and their roots were dipped in a freshly prepared conidial suspension of *F. moniliforme* for 3hrs before transplanting. The inoculated seedlings were then transplanted in the field.

Table 12: Germplasm lines evaluated against foot rot disease under field condition

Sl.no	Entry	Designation
1	RYT-BT-1-1	6001*
2	RYT-BT-1-2	6002
3	RYT-BT-1-3	6004
4	RYT-BT-1-4	RYT-BT-6
5	RYT-BT-1-5	RYT-BT-10
6	RYT-BT-1-6	RYT-BT-15
7	RYT-BT-1-7	RYT-BT-16
8	RYT-BT-1-8	AVT-IBT-2101
9	RYT-BT-1-9	AVT-IBT-2103
10	RYT-BT-1-10	AVT-IBT-2104
11	RYT-BT-1-11	AVT-IBT-2106
12	RYT-BT-1-12	CR-2007
13	RYT-BT-1-13	Pusa Punjab Bas-1509
14	RYT-BT-1-14	Pusa Bas 1121
15	RYT-BT-1-15	Punjab Bas 2
16	RYT-BT-1-16	Bas 370
17	RYT-BT-1-17	Bas 386
18	RYT-BT-1-18	Punjab Basmati-3
19	RYT-BT-1-19	Sharbati
20	RYT-BT-1-20	PB Mehak-1
21	RYT-BT-1-21	Dewe Gowda
22	RYT-BT-2-1	6291-1
23	RYT-BT-2-2	6295-2
24	RYT-BT-2-3	6297-1
25	RYT-BT-2-4	6298-3
26	RYT-BT-2-5	6305-2
27	RYT-BT-2-6	6306-1
28	RYT-BT-2-7	6307-2
29	RYT-BT-2-8	6308-2
30	RYT-BT-2-9	6328-2
31	RYT-BT-2-10	6329
32	RYT-BT-2-11	6330
33	RYT-BT-2-12	6331-1

34	RYT-BT-2-13	6396
35	RYT-BT-2-14	-
36	RYT-BT-2-15	6670-1
37	IYT-BT-101	6283-1
38	IYT-BT-102	6284-1
39	IYT-BT-103	6285-2
40	IYT-BT-104	6288
41	IYT-BT-105	6289-2
42	IYT-BT-106	6290-2
43	IYT-BT-107	6292-1
44	IYT-BT-108	6294
45	IYT-BT-109	6300-1
46	IYT-BT-110	6301-2
47	IYT-BT-111	6302-1
48	IYT-BT-112	6303-3
49	IYT-BT-113	6304-1
50	IYT-BT-114	6306-4
51	IYT-BT-115	6308-1
52	IYT-BT-116	6310-1
53	IYT-BT-117	6310-3
54	IYT-BT-118	6314-2
55	IYT-BT-119	6315-2
56	IYT-BT-120	6318-3
57	IYT-BT-121	6321-2
58	IYT-BT-122	6322
59	IYT-BT-123	6338-1
60	IYT-BT-124	6339
61	IYT-BT-125	6342-2
62	IYT-BT-126	6344-2
63	IYT-BT-127	6346
64	IYT-BT-128	6350-2
65	IYT-BT-129	6356-1
66	IYT-BT-130	6357-2
67	IYT-BT-131	6361
68	IYT-BT-132	6362-2

69	IYT-BT-133	6366
70	IYT-BT-134	6372-1
71	IYT-BT-135	6377-2
72	IYT-BT-136	6385
73	IYT-BT-137	6386-1
74	IYT-BT-138	6387
75	IYT-BT-139	6388-4
76	IYT-BT-140	6389-1
77	IYT-BT-141	6393
78	IYT-BT-142	6397
79	IYT-BT-143	6400-1
80	IYT-BT-144	6401-2
81	IYT-BT-145	6405-1
82	IYT-BT-146	6411
83	IYT-BT-147	6447
84	IYT-BT-148	-
85	IYT-BT-149	6451-3
86	IYT-BT-150	6456-1
87	IYT-BT-151	6458-1
88	IYT-BT-152	6459-1
89	IYT-BT-153	6461-2
90	IYT-BT-154	6463
91	IYT-BT-155	6465
92	IYT-BT-156	6466-1
93	IYT-BT-157	6482-2
94	IYT-BT-158	6485
95	IYT-BT-159	6488-2
96	IYT-BT-160	6489-3
97	IYT-BT-161	6490-2
98	IYT-BT-162	6500
99	IYT-BT-163	6508
100	IYT-BT-164	6521-1
101	IYT-BT-165	6525-2
102	IYT-BT-166	6533-2
103	IYT-BT-167	6537-4

104	IYT-BT-168	6538-2
105	IYT-BT-169	6541-1
106	IYT-BT-170	6549-1
107	IYT-BT-171	6552-2
108	IYT-BT-172	6553-2
109	IYT-BT-173	6554-2
110	IYT-BT-174	6594
111	IYT-BT-175	6612
112	IYT-BT-176	6638
113	IYT-BT-177	6653
114	IYT-BT-178	6662
115	IYT-BT-179	6665
116	IYT-BT-180	6669
117	32799 Bulk	PAU 3245-42
118	32800-Bulk	PAU 3245-43
119	32804-Bulk	PAU 3245-47
120	32807-Bulk	PAU 3245-50
121	32808-Bulk	PAU 3245-51
122	GSK-27	IR 74718-147-1-1-3
123	GSK-65	IR 75478-55-3
124	GSK-95	IR 75479-46-1-2
125	GSK-98	IR 75479-59-3-3
126	GSK-133	IR 75482-140-2-3
127	GSK-139	IR 75483-33-3-2
128	GSK-140	IR 75483-50-3-1
129	GSK-188	IR 75490-337-2-1
130	GSK-194	IR 71735-6-3-3
131	GSK-196	IR 44699-21-1-3-4
132	2484 ctk	IR 75482-135-2-3
133	2485 Ctk	-
134	2486 Ctk	-

3.9.2 Screening in nursery

All the lines were also evaluated in nursery during 2014. The seeds were dipped in spore suspension (4×10^4spores/ml) of *F. moniliforme* for 3hrs and were sown in nursery in the month of June' 2014. Observations on disease incidence were recorded 15 and 30 days after

sowing. The per cent disease incidence (PDI) was calculated as follows (Teng and James 2001):

$$PDI = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \text{ x } 100$$

The germplasm lines were classified in different categories on the basis of disease scale for foot rot disease of rice (Khan *et al* 2000, Ghazanfar *et al* 2013, Fiyaz *et al* 2014).

Table 13: Disease scale used for evaluation against foot rot disease of rice

Disease incidence (%)	**Host Response**
0-10	Highly resistant
11-20	Resistant
21-40	Moderately resistant
41-60	Moderately susceptible
61-80	Susceptible
Above 80	Highly susceptible

CHAPTER-IV

RESULTS AND DISCUSSIONS

The results of various experiments conducted for investigations on foot rot (*Fusarium moniliforme* Sheld.) of basmati rice with reference to survival, host ranges, variability of pathogen and host-resistance are presented in this chapter under different headings as follow.

4.1 Symptoms of foot rot caused by *Fusarium moniliforme*

Different types of symptoms of disease were observed under field conditions on the seedlings/ plants raised from infected seed.

4.1.1 Elongation of seedlings in nursery

Abnormal elongation of the seedlings was observed (Plate1) in nursery after 15-20 days of sowing. The seedlings which emerged from infected seed were taller and pale in color than the normal one.

4.1.2 Foot rot symptoms in nursery

The elongated seedlings started sudden wilting and drying after few days followed by rotting of lower portion of stem starting from soil level. Whitish pink growth of pathogen consisting of mycelium, and conidia became visible.

4.1.3 Elongation of plants after transplanting

Some plants showed elongation after transplanting in the field after 10-30 days. Tillering was also significantly reduced. In some plants all the tillers were infected whereas in few cases only few tillers showed elongation and foot rot symptoms.

4.1.4 Foot rot symptoms in field after transplanting

Thin plants with yellowish green leaves and pale green flag leaves were observed. Whitish to pinkish fungal growth on lower part of the plant was observed (Plate1.6). In later stage, partial or complete dying of the foot rot infected plants was observed (Plate 1.5).

4.1.5 Stunting of seedlings

Some seedlings remained stunted as compare to the normal ones. Numbers of stunted plants are few as compared to the elongated ones. These are killed within 1-2 days.

4.1.6 Adventitious roots

Production of adventitious roots on lower nodes of some infected plants was observed (Plate1.4). Adventitious roots do not appear always but sometimes it appears in the internodes.

4.1.7 Sterile ears

Very few plants showed elongation symptoms late in the season produced sterile tillers. (Plate 1.7).

4.2 Disease cycle of bakanae

Fusarium moniliforme causing foot rot of rice is primarily a seed-borne pathogen. However, it can also become soil borne if chlamydospores, sclerotia etc are produced. In the

Plate 1: Symptoms of Foot rot disease observed in nursery and field

1.1 Infected plants taller than normal plants in seedbed

1.2 Infected plants taller than normal plants in transplanted field

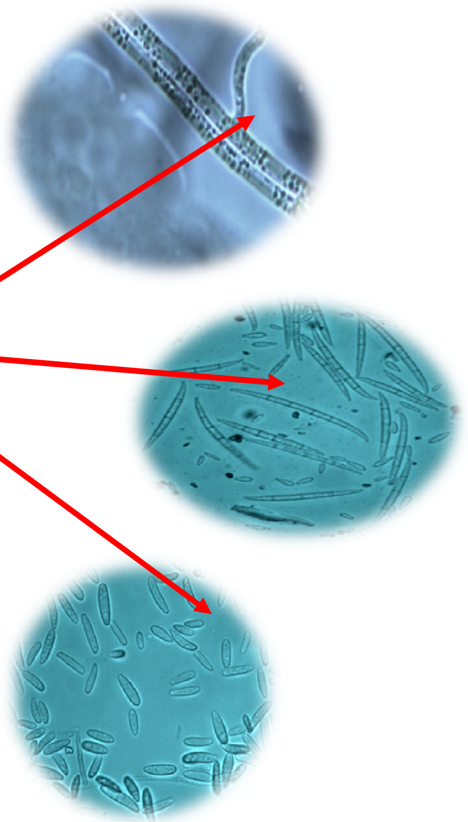
1.3 Thin plants with yellowish green leaves and pale green flag leaves

1. 4 Formation of adventitious roots

1.5 Partial dying of the foot rot infected plants

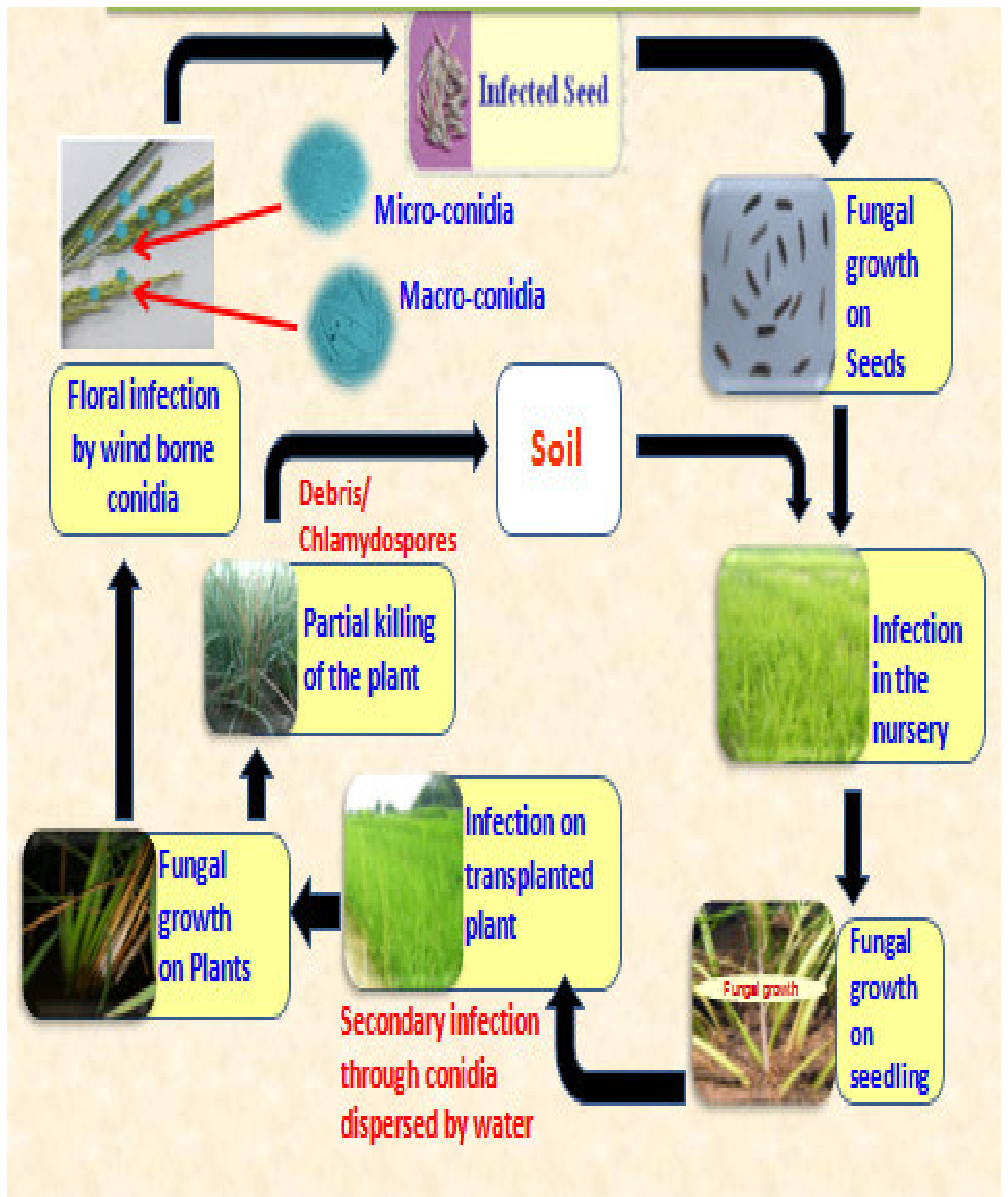
1.6 Partially filled sterile or empty grains on surviving plant at maturity

1.7 Stunted symptoms observed in infected plants



1.8 Foot rot, whitish or pinkish growth on lower part

Plate 2. Disease cycle of Foot rot caused by *F. moniliforme* in rice



present investigation, it has been revealed that it doesn't produce chlamydospores under Punjab conditions. The mycelium and conidia on infected straw does not survive for longer period of time till next season. The conidia present in/on the seed germinate and cause infection to young seedlings through roots. The seedlings emerging from infected seed either showed elongation or stunting. The seedlings emerging from infected seed die after few days. The elongated seedlings started wilting and drying after few days. On the lower portion of the infected seedlings lot of purplish white mycelium growth producing conidia was visible and then the seedlings die. The conidia from infected seedlings are spread in the nursery bed and cause secondary infection to the injured roots at the time of uprooting of nursery for transplanting. The pathogen grew systemically and these infected seedlings then produce symptoms after 10-15 days of transplanting. Most of the infected seedlings produce symptoms upto 25-30 days after transplanting. Then after few days the elongated plants start wilting and drying. The pinkish white mycelium producing conidia becomes visible on the portion of tillers. The conidia then become wind borne and cause infection to the developing grains at the time of flowering stage.

4.3 Characterization of Cultural traits of *Fusarium moniliforme* causing foot rot disease

Cultural characteristics of 38 isolates were studied on three different media i.e., Potato Dextrose Agar (PDA), Soil Extract Agar (SEA) and Spezieller Nahrstoffarmer Agar (SNA) in Petri dishes. Different cultural characteristics like colony elevation, texture, pigment production both on surface and reverse of the culture plate, zonations and exudations on culture were recorded after 8 days of incubation at 25 ± 2^0 C. Based on these traits, cultural variations among the 38 isolates were distinctly visible on PDA and SEA. But, the variation was not obvious on SNA media.

Colony elevation and texture were studied for all the isolates on the basis of whether the colony was highly raised or medium raised or slightly raised (Table 14). It was observed that colony elevation varied for all the isolates on the two media PDA and SEA. The isolate FR12 produced a type of colony called crateriform colony i.e., depressed at the centre as compared to other portion of the culture on the Petri plate. This characteristic has been observed on both PDA and SEA media. Texture of the colony was cottony and fluffy on PDA and SEA. However, it was denser on PDA as compared to the other two media. The colony was sparse and thin on SNA. Besides the colony characters, production of exudation and zonation were also observed on PDA and SEA but exudations were commonly observed on SEA media. Not all isolates produced exudations and zonations. The isolate FR 24, FR25 and FR33 produced coloured exudations on PDA and the isolate FR24 produced V-shaped sector. Exudations and sectors were more distinctly visible on SEA media. The isolates FR 9 and 24 showed V-shaped sectors on SEA. Other feature like production of concentric rings was observed for FR 17 and FR 18 on SEA media and such features were not observed on other

Table 14. Variability in cultural characteristics of different isolates of *Fusarium moniliforme* on Potato Dextrose Agar (PDA) and Soil Extract Agar (SEA) media

Id. no	Media type	Cultural characteristics		Pigmentation		Zonation and exudations
		Colony elevation	Texture	Surface color	Color on reverse	
FR 1	PDA	Highly raised	Fluffy cottony	Light coral at centre and white towards periphery	Cream	-
	SEA	Medium raised	Fluffy cottony even	White	Cream	-
FR 2	PDA	Slightly raised	Cottony	Velvet maroon at center and white towards periphery	Velvet maroon at center and white towards periphery	-
	SEA	Slightly raised	Fluffy cottony	Milky white	Cream	-
FR 3	PDA	Highly raised	Fluffy cottony	Pearl	Cream	-
	SEA	Medium raised	Fluffy cottony	Pearl	Cream	
FR 4	PDA	Slightly raised	Fluffy	Pink bow pigmentation at center and white towards periphery	Pink bow pigmentation at center and white towards periphery	-
	SEA	Slightly raised, centre raised	Fluffy cottony even	White, Pink tinge in the centre	Cream	-
FR 5	PDA	Slightly raised	Fluffy cottony	White	Cream	-
	SEA	Highly raised Centre raised	Fluffy cottony even	White	Cream	
FR 6	PDA	Medium raised	Fluffy cottony	White	Cream	-
	SEA	Medium raised Centre is raised	Fluffy cottony	Milk White	Cream	-
FR 7	PDA	Medium flat	Cottony	Rose	Tulip pink becoming lighter till margin	-
	SEA	Flat, raised at centre	Fluffy cottony	Rose	Tulip pink becoming lighter till margin	-
FR 8	PDA	Slightly raised	Fluffy cottony	Egg plant at centre and white towards periphery	Egg plant at centre and white towards periphery	-
	SEA	Highly raised	Fluffy cottony even	Pearl at centre and white towards periphery	Cream	-
FR 9	PDA	Flat	Cottony	Milk white	Cream	-
	SEA	Medium raised	Cottony fluffy	Milk white	Cream	Light V-shaped

			uneven			sectors Watery/ transparent exudation
FR 10	PDA	Slightly raised	Fluffy cottony	Antique white at centre and milk white towards periphery	Cream	-
	SEA	Slightly raised	Cottony loose growth even	White	Cream	-
FR 11	PDA	Slightly raised	Fluffy cottony	White	Cream	-
	SEA	Highly raised at the centre	cottony	White	Cream	-
FR 12	PDA	Raised (Crateriform colony depressed at the center)	Fluffy cottony	White	Peach	-
	SEA	Highly Raised (Crateriform colony depressed at the center)	Fluffy cottony uneven	Milk White	Cream	-
FR 13	PDA	Slightly raised	Little fluffy cottony	Dull purple at centre and white towards periphery	Cream	-
	SEA	Flat	Cottony Even	Antique tinge at the centre white towards periphery	Cream	-
FR 14	PDA	Slightly raised	Fluffy cottony, filamentous	White	Cream	-
	SEA	Highly raised	Fluffy cottony, even	White	Cream	-
FR 15	PDA	Flat	Even cottony	Peach tinge at centre and milk white towards periphery	Cream	-
	SEA	Flat	Even cottony	Peach tinge at centre and milk white towards periphery	Cream	-
FR 16	PDA	Raised	Fluffy cottony	Pearl at centre and milk white towards periphery	Cream	-
	SEA	Raised	Fluffy cottony	Pearl at centre and milk white towards periphery	Cream	-
FR 17	PDA	Slightly raised	Little fluffy cottony	Pearl	Cream	-

	SEA	Medium raised	Fluffy cottony	Seashell at centre and milk white towards periphery	Cream	1-2 concentric rings towards periphery watery/ transparent exudations
FR 18	PDA	Raised at center (Umbonate)	Fluffy cottony, dense growth	Tulip pink	Tulip pink	-
	SEA	Raised	Fluffy cottony, even growth	Pinkish tinge at centre, white	Cream	Concentric rings
FR 19	PDA	Flat	Compact cottony	White	Cream	-
	SEA	Highly raised at centre	Aerial fluffy cottony at centre, flat towards periphery	White	Cream	-
FR 20	PDA	Raised	Fluffy cottony	White	Cream	
	SEA	Medium raised	Fluffy cottony	White	Cream	
FR 21	PDA	Raised	Fluffy cottony	Pink rose	Pink rose	-
	SEA	Flat (centre Raised)	Cottony, even texture, upright mycelium	White	Cream	-
FR 22	PDA	Raised	Fluffy cottony	White	Cream	-
	SEA	Slightly raised	fluffy cottony, even	Milk white	Cream	-
FR 23	PDA	Medium raised	Little fluffy cottony	Milk white	Cream	-
	SEA	Flat with centre Raised	Even Cottony	Dull purple	Cream	-
FR 24	PDA	Raised	Cottony	White	Cream	V shaped sectors (sectorization
	SEA	Raised	Cottony	White	Cream	V shaped sectors (sectorization)
FR 25	PDA	Medium raised	Cottony	White	Cream	Red Fox coloured
	SEA	Raised at centre	Fluffy cottony even		Cream	-
FR 26	PDA	Raised	Fluffy cottony	Rose	Peach	-
	SEA	Highly raised (Crateriform-colony depressed at center)	Cottony	Milk White	Cream	Watery/ transparent exudation
FR 27	PDA	Highly raised (Crateriform-colony depressed at center)	Fluffy cottony	Milk White	Cream	-

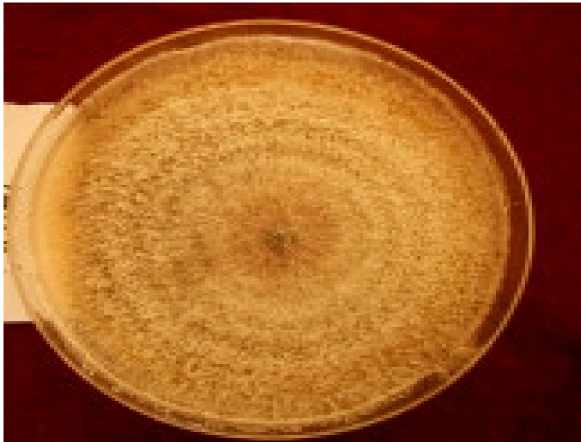
	SEA	Highly Raised at the centre	Cottony	White	Cream	-
FR 28	PDA	Raised	Fluffy cottony, dense growth	Antique White	Cream	-
	SEA	Raised	Cottony	Antique White	Cream	
FR 29	PDA	Flat	Fluffy cottony, aerial mycelium	White	Cream	-
	SEA	Slightly raised	Fluffy cottony, dense mycelium, uneven	White	Cream	-
FR 30	PDA	Slightly raised	Fluffy cottony, aerial mycelium	Milk White	Cream	-
	SEA	Slightly raised	Fluffy cottony, aerial mycelium	Milk White	Cream	-
FR31	PDA	Raised	Fluffy cottony	Dull purple Pigmentation max. At centre and diluting out towards periphery with white	Midnight	-
	SEA	Flat	Cottony, even, loose growth	White	Cream	-
FR32	PDA	Medium Flat	Cottony	Pink bow near centre and white towards periphery	Pink bow near centre	-
	SEA	Flat	Slightly fluffy cottony even texture	Milk white	Cream	-
FR33	PDA	Slightly raised	Cottony	Velvet maroon	Velvet maroon white towards periphery	Maroon coloured
	SEA	Flat, slightly raised at the centre	Cottony Even texture	White	Cream	-
FR34	PDA	Medium raised	Fluffy Cottony	Pink bow around centre which diffuses to lighter shade periphery towards	Cream	-
	SEA		Cottony Fluffy even	White	Blanched almond	-
FR35	PDA	Raised	Fluffy cottony	White	Cream	-
	SEA	Slightly raised at centre	Thin not Fluffy cottony growth, even texture	White	Cream	-
FR36	PDA	Medium raised	Fluffy cottony	White	Cream	-
	SEA	Centre slightly raised at centre,	Fluffy cottony growth, even	Milk white	Cream	-

		flat	texture			
FR37	PDA	Slightly raised	Fluffy cottony	Deep peach at centre and white towards periphery	Cream	-
	SEA	Flat	Cottony slightly fluffy at periphery even	White	Cream	-
FR38	PDA	Slightly raised	Fluffy cottony aerial mycelium	Milky white	Cream	-
	SEA	Raised at centre	Thin cottony growth, even	White	Cream	-

media. Watery and transparent exudations were produced by the isolates FR 9, 17 and 26 on SEA. Colored exudations were produced by the two isolates FR 25 (red fox colored) and FR 33 (maroon colored) on PDA media but no such observations were recorded on SEA. Cultural variability of *Fusarium moniliforme* on PDA, SEA and SNA Media, colony texture, elevation, pigmentation, exudations and sectorization were presented in plates 3 and 4.

Colony color was also observed on all the three media. The color of the culture on the media was judged according to the color chart. Comparative variability in colony colour on both surface and reverse of the plate was observed on PDA and SEA. However, no variability was observed on SNA. Generally, the colony color of all the 38 isolates vary from whitish to pinkish in color on the surface. More intense pigments were observed on PDA as compared to SEA. The culture on SEA media mostly showed white to milky white colour. However, on PDA the culture produced visibly distinct pigments at the centre which diffuses out to the periphery. Not all the isolates produced pigment on the reverse. The isolates FR 2, 4, 7, 12, 18, 21, 25, 26, 31, 32, 33 and 34 produced different colors on reverse but the remaining isolates produced only cream color on reverse. On the basis of the color, they produced on the culture, it can be concluded that the isolates vary in the production of colony color ranging between white to pink (Table 14). The findings corroborate to the findings of Leslie and Summerell (2006), Ilija *et al* (2009) and Pannu *et al* (2013) who also reported that pigmentation in the agar varies and ranged from no pigmentation or grayish orange in some isolates to violet grey, dark violet or dark magenta (almost black) in others. Atukwase *et al* (2012) also reported that colonies on PDA generally produced white/ purple color interspersed with rings of different shades of purple/ white colors. Kaur *et al* (2014) also observed the colony color of *Fusarium* spp causing foot rot to be white to purplish. From the cultural characteristics observed on different media, it revealed that different culture media influenced the growth and pigmentation of the culture as in case of *Fusarium moniliforme* KUMBF1201 reported by Pradeep *et al* (2013) on eight solid media (PDA, MA, RBA, OMA, YMA, CZA, SDA and NA). The findings also agreed with the observations of Pannu *et al* (2013) who reported that different media and temperature effect the growth and colony

Plate 3. Cultural variability of *Fusarium moniliforme* on PDA, SEA and SNA Media



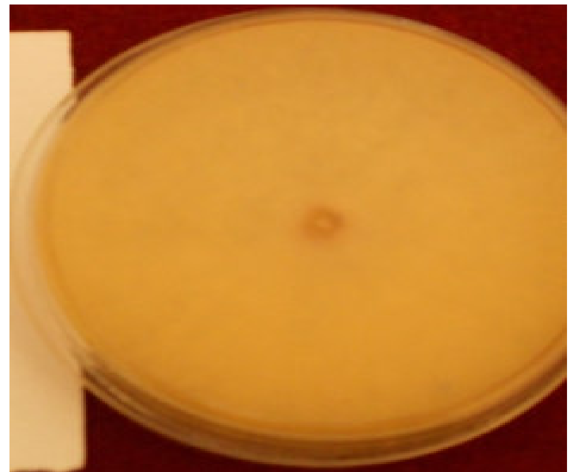
3.1. Culture on PDA (surface)

3.5. Reverse side on PDA

3.2. Culture on SEA (surface)

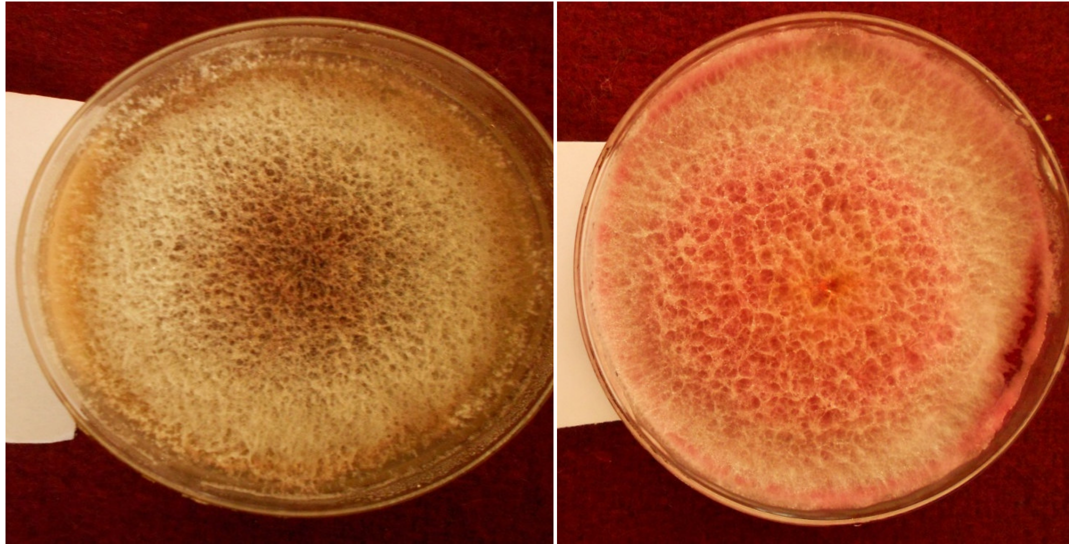
3.6. Reverse side on SEA

3.3. Culture on SNA(surface)

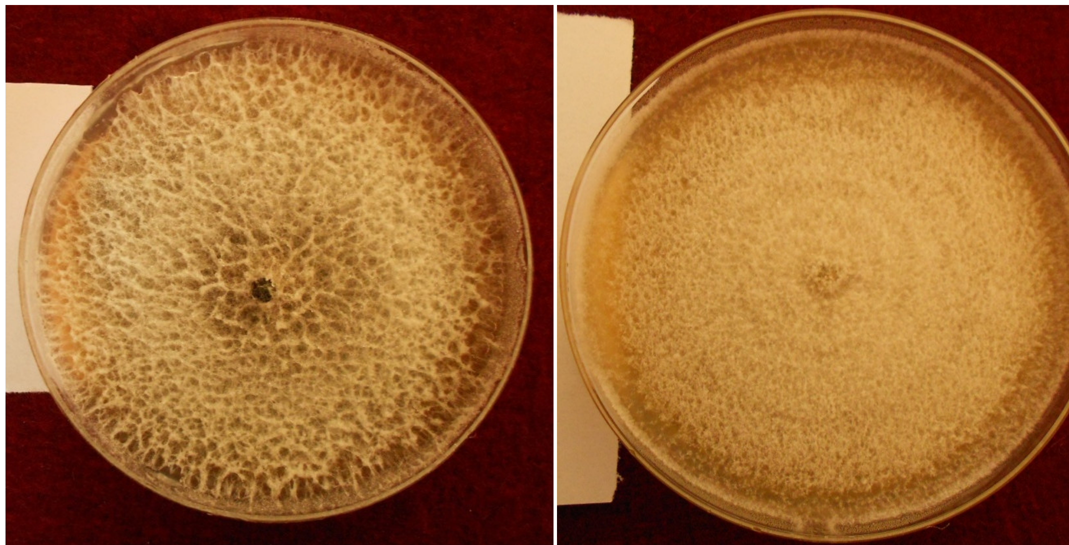


3.7 Reverse side on SNA

Plate 4. Cultural variability showing colony texture, elevation, pigmentation, exudations and sectorization

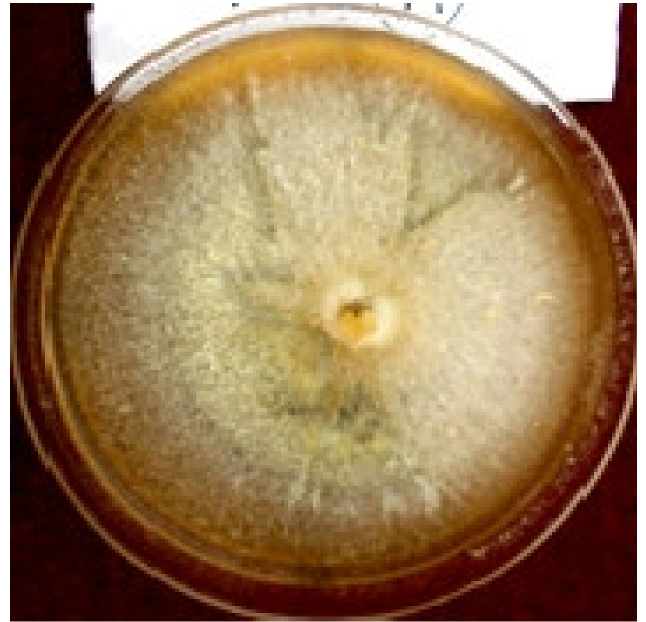


4.1 Appearance of pigmentation in the culture



4.2 Fuffy Cottony growth **4.3 Cottony growth**

4.4 Appearance of exudations



4.5 Appearance of sectors

4.6 Appearance of rings in the culture

characters of *Fusarium moniliforme* causing foot rot of basmati rice.

The isolates also showed variation in colony diameter and growth rate on all the three media. The variability among the isolates has been observed grown on the same media and also between different media. The best growth was observed on PDA (Table 15), followed by SEA (Table 16) and SNA (Table 17). The growth rate varied between 12.28-25.86-mm/ 48 hrs on PDA, between 9.09-19.59 mm/ 48 hrs on SEA and between 5.97- 16.44 mm/ 48hrs on SNA media. Duncan Multiple Range test indicated that most of the isolates were significantly different (P=0.05).

Table 15: Variability in colony diameter and growth rate of *Fusarium moniliforme* on Potato Dextrose Agar media

I.d. no.	Colony diameter (mm/day)				Growth rate (mm/48hrs)			
	2nd day	4th day	6th day	8th day	4-2day	6-4day	8-6day	Average Growth rate
FR 1	19.00	38.25	60.67	78.00	19.25	22.42	17.33	19.67 h-k
FR 2	18.58	37.50	62.58	80.50	18.92	25.08	17.92	20.64 j-l
FR 3	18.53	38.37	57.70	74.50	19.84	19.33	16.80	18.66 e-j
FR 4	18.92	38.92	60.42	76.33	20.00	21.50	15.91	19.14 f-k
FR 5	18.92	40.75	59.42	74.92	21.83	18.67	15.50	18.67 e-j
FR 6	13.64	23.89	42.39	54.55	10.25	18.50	12.16	13.64 ab
FR 7	15.42	29.33	46.33	63.00	13.91	17.00	16.67	15.86 cd
FR 8	19.57	36.52	53.77	68.00	16.95	17.25	14.23	16.13 cd
FR 9	5.18	19.16	41.58	53.00	13.98	22.42	11.42	15.94 cd
FR 10	18.83	34.66	50.7	69.62	15.83	16.04	18.92	16.93 de
FR 11	30.58	42.17	59.92	71.75	11.59	17.75	11.83	13.72 ab
FR 12	9.00	24.16	47.66	64.00	15.16	23.50	16.34	18.33 e-i
FR 13	5.11	20.30	41.42	58.00	15.19	21.12	16.58	17.63 d-g
FR 14	35.83	47.42	65.17	76.67	11.59	17.75	11.50	13.61ab
FR 15	12.99	28.82	48.14	65.42	15.83	19.32	17.28	17.48 d-f
FR 16	41.18	50.43	66.93	78.01	9.25	16.50	11.08	12.28 a
FR 17	2.83	18.99	43.49	61.58	16.16	24.50	18.09	19.58 g-k
FR 18	22.00	44.00	68.08	82.08	22.00	24.08	14.00	20.03 i-l
FR 19	19.42	42.33	65.92	84.75	22.91	23.59	18.83	21.77 mn
FR 20	25.46	38.05	56.8	69.36	12.59	18.75	12.56	14.63 bc
FR 21	16.58	33.00	53.75	69.08	16.42	20.75	15.33	17.50 d-f
FR 22	19.92	42.25	66.58	82.25	22.33	24.33	15.67	20.78 k-l
FR 23	20.42	42.75	65.08	82.33	22.33	22.33	17.25	20.64 j-l

FR 24	12.58	36.49	61.5	82.33	23.91	25.01	20.83	23.25 ^{no}
FR 25	7.16	35.41	65.75	84.75	28.25	30.34	19.00	25.86 ^{p}
FR 26	16.17	29.33	44.58	56.83	13.16	15.25	12.25	13.55 ^{ab}
FR 27	18.13	32.88	51.79	66.88	14.75	18.91	15.09	16.25 ^{cd}
FR 28	18.67	36.83	56.17	73.92	18.16	19.34	17.75	18.42 $^{e-i}$
FR 29	18.76	31.35	50.10	61.67	12.59	18.75	11.57	14.30 ^{bc}
FR 30	39.26	51.76	70.51	82.08	12.50	18.75	11.57	14.27 ^{bc}
FR 31	20.00	41.58	61.75	78.67	21.58	20.17	16.92	19.56 $^{g-k}$
FR 32	23.39	45.42	66.83	81.67	22.03	21.41	14.84	19.43 ^{op}
FR 33	16.83	35.5	56.17	70.00	18.67	20.67	13.83	17.72 $^{d-h}$
FR 34	15.85	30.35	45.77	59.10	14.5	15.42	13.33	14.42 ^{bc}
FR 35	24.33	43.50	64.5	76.00	19.17	21.00	11.50	17.22 $^{d-f}$
FR 36	19.67	39.25	62.5	79.42	19.58	23.25	16.92	19.92 $^{i-l}$
FR 37	19.10	36.10	56.76	75.00	17.00	20.66	18.24	18.63 $^{e-j}$
FR 38	13.36	23.52	42.07	54.32	10.16	18.55	12.25	13.65 ^{ab}

*Data followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 16. Variability in colony diameter and growth rate of *Fusarium moniliforme* on Soil Extract Agar media

ld.no.	Colony diameter (mm/day)				Growth Rate (mm/48hrs)			
	2nd	4th	6th	8th	4th-2nd	6-4th	8-6th	Average Growth rate
FR 1	13.50	47.00	60.52	70.17	33.50	13.52	9.65	18.89 $^{n-p}$
FR 2	10.42	30.00	50.15	66.15	19.58	20.15	16.01	18.58 $^{m-p}$
FR 3	18.00	36.00	56.00	74.09	18.00	20.00	18.09	18.70 $^{n-p}$
FR 4	19.33	38.48	61.25	73.83	19.15	22.77	12.58	18.17 $^{l-o}$
FR 5	14.33	30.17	47.67	63.67	15.83	17.50	16.00	16.44 $^{g-i}$
FR 6	8.67	16.50	28.67	44.67	7.83	12.17	16.00	12.00 ^{c}
FR 7	11.42	20.58	32.50	47.50	9.17	11.92	15.00	12.03^{c}
FR 8	14.25	37.92	47.58	55.50	23.67	9.67	7.92	13.75 ^{e}
FR 9	15.17	30.33	44.17	55.83	15.17	13.83	11.67	13.56 ^{e}
FR 10	10.00	26.50	44.00	56.67	16.50	17.50	12.67	15.56 ^{fg}
FR 11	9.33	19.83	37.58	46.25	10.50	17.75	8.67	12.31 ^{cd}
FR 12	16.83	29.20	51.50	69.61	12.37	22.30	18.11	17.59 $^{j-m}$
FR 13	17.33	32.67	47.92	61.75	15.33	15.25	13.83	14.81^{f}
FR 14	14.17	27.17	45.58	53.50	13.00	18.42	7.92	13.11 ^{de}
FR 15	13.33	27.23	39.13	61.91	13.89	11.90	22.78	16.19 ^{gh}

FR 16	12.17	21.00	28.60	39.44	8.83	7.60	10.84	9.09 ^{a}
FR 17	16.33	31.69	49.19	68.53	15.36	17.50	19.33	17.40 $^{i-l}$
FR 18	12.75	24.50	47.11	66.30	11.75	22.61	19.19	17.85 $^{k-n}$
FR 19	19.33	40.42	63.28	78.11	21.08	22.86	14.83	19.59 ^{p}
FR 20	14.42	28.50	47.11	55.94	14.08	18.61	8.83	13.84^{e}
FR 21	13.00	33.67	50.00	65.58	20.67	16.33	15.58	17.53^{j-l}
FR 22	14.17	32.17	53.69	70.43	18.00	21.53	16.73	18.75 $^{n-p}$
FR 23	15.17	34.53	51.30	67.44	19.36	16.78	16.14	17.43 $^{i-l}$
FR 24	19.50	35.50	54.50	69.17	16.00	19.00	14.67	16.56 $^{h-j}$
FR 25	20.25	51.08	72.92	78.92	30.83	21.83	6.00	19.56 ^{p}
FR 26	16.25	25.42	41.39	56.86	9.17	15.97	15.47	13.54 ^{e}
FR 27	10.33	21.61	39.00	45.67	11.28	17.39	6.67	11.78 ^{c}
FR 28	19.92	35.42	54.75	70.17	15.50	19.33	15.42	16.75 $^{h-j}$
FR 29	11.83	19.95	35.40	43.35	8.11	15.46	7.94	10.50 ^{b}
FR 30	10.17	22.28	33.55	40.44	12.11	11.28	6.89	10.09 ^{b}
FR 31	9.67	23.14	40.12	56.17	13.47	16.98	16.05	15.50 ^{fg}
FR 32	18.50	39.25	62.58	76.58	20.75	23.33	14.00	19.36 ^{p}
FR 33	17.50	27.15	37.67	52.47	9.65	10.52	14.80	11.66 ^{c}
FR 34	15.50	31.08	45.33	52.72	15.58	14.25	7.38	12.41 ^{cd}
FR 35	17.82	46.43	54.64	68.53	28.62	8.21	13.88	16.90 $^{h-k}$
FR 36	16.57	48.83	67.75	73.83	32.27	18.92	6.08	19.09 ^{op}
FR 37	12.67	26.17	57.92	68.50	13.50	31.75	10.58	18.61 $^{m-p}$
FR 38	12.41	27.42	38.67	48.53	15.00	11.25	9.86	12.04 ^{c}

Table 17. Variability in colony diameter and growth rate of *Fusarium moniliforme* on Spezieller Nahrstoffarmer Agar media

I.d.no.	Colony diameter (mm/day)				Growth rate (mm/48 hrs)			
	2nd	**4th**	**6th**	**8th**	**4th-2nd**	**6th-4th**	**8-6th**	**Average growth rate**
FR 1	16.50	27.33	48.00	58.13	10.83	20.67	10.13	13.88 ^{n}
FR 2	13.76	28.10	38.79	54.79	14.34	10.70	16.00	13.68 ^{mn}
FR 3	13.73	24.13	36.00	53.00	10.40	11.87	17.00	13.09^{l-n}
FR 4	18.83	28.39	50.98	65.13	9.56	22.58	14.15	15.43^{o}
FR 5	14.67	26.50	37.83	52.50	11.83	11.33	14.67	12.61^{i-k}
FR 6	14.83	23.19	34.17	39.17	8.35	10.98	5.00	8.11^{b}
FR 7	15.00	25.67	36.27	42.31	10.67	10.60	6.04	9.10 ^{c}
FR 8	19.50	32.43	37.54	53.60	12.93	5.11	16.06	11.37 $^{e-i}$

FR 9	13.50	27.50	39.83	48.83	14.00	12.33	9.00	11.78 $^{e-k}$
FR 10	13.00	23.67	36.17	49.33	10.67	12.50	13.17	12.11 $^{h-k}$
FR 11	14.50	26.33	37.17	47.17	11.83	10.83	10.00	10.89 $^{d-f}$
FR 12	17.67	27.61	44.55	59.55	9.93	16.94	15.00	13.96 ^{n}
FR 13	14.50	23.83	37.17	47.00	9.33	13.33	9.83	10.83 ^{de}
FR 14	14.33	23.50	28.50	38.17	9.17	5.00	9.67	7.94 ^{b}
FR 15	13.50	26.38	40.23	54.50	12.88	13.86	14.27	13.67 ^{mn}
FR 16	18.33	24.02	31.35	38.18	5.68	7.33	6.83	6.62 ^{a}
FR 17	21.00	41.17	54.17	66.33	20.17	13.00	12.17	15.11 ^{o}
FR 18	11.11	32.50	47.50	58.20	21.40	15.00	10.70	15.70 ^{op}
FR 19	12.50	35.553	53.72	62.28	23.05	18.17	8.56	16.59 ^{p}
FR 20	13.00	23.98	36.14	48.56	10.98	12.16	12.43	11.85 $^{f-k}$
FR 21	14.93	26.83	39.00	53.00	11.90	12.167	14.00	12.69 $^{i-k}$
FR 22	13.00	27.83	39.167	51.50	14.83	11.33	12.33	12.83 $^{k-m}$
FR 23	14.50	29.00	40.167	49.67	14.50	11.17	9.50	11.72 $^{e-j}$
FR 24	13.67	23.00	37.23	48.50	9.33	14.23	11.27	11.61 $^{e-i}$
FR 25	13.00	37.00	54.13	62.33	24.00	17.13	8.20	16.44 ^{p}
FR 26	12.67	23.03	36.40	42.81	10.36	13.39	6.39	10.05 ^{d}
FR 27	13.83	22.667	35.83	44.84	8.83	13.17	9.01	10.34 ^{d}
FR 28	16.5	23.17	34.83	49.50	6.67	11.67	14.67	11.00 $^{d-g}$
FR 29	16.17	21.22	31.95	34.08	5.05	10.73	2.13	5.97 ^{a}
FR 30	13.50	17.12	25.27	31.49	3.63	8.13	6.23	6.00 ^{a}
FR 31	10.50	26.03	38.39	42.92	15.53	12.36	4.52	10.81 ^{de}
FR 32	10.50	27.17	41.83	58.33	16.67	14.67	16.50	15.94 ^{op}
FR 33	16.33	23.50	36.50	52.17	7.17	13.00	15.67	11.94 $^{g-k}$
FR 34	15.833	26.17	39.83	54.00	10.33	13.67	14.17	12.72 $^{j-m}$
FR 35	13.67	27.83	43.17	54.50	14.17	15.33	11.33	12.89 $^{k-m}$
FR 36	17.67	33.25	55.58	65.19	15.58	22.33	9.61	15.84 ^{op}
FR 37	16.00	27.00	54.83	62.94	11.00	27.83	8.11	15.65 ^{op}
FR 38	14.167	23.50	43.33	49.32	9.33	19.83	5.99	11.72 $^{e-j}$

On the basis of the growth rate on PDA and SEA, the isolates were grouped as slow, medium, fast and very fast growers (Table 18). The growth was dense and fluffy for both PDA and SEA, moderate and not fluffy on SNA. The studies revealed that variation in the culture media influenced the growth and pigmentation of the culture (Pradeep *et al* 2013, Pannu *et al*

2013). PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelial growth (Pannu *et al* 2013). Pradeep *et al* (2013) also revealed that PDA is the best medium for maximum mycelial growth and requirements for carbon, nitrogen, sulfur and energy and pigment production.

Table 18. Grouping of the isolates on the basis of growth rate on Potato dextrose agar and Soil Extract Agar

Growth range	Group	Isolates	
		PDA	**SEA**
less than 10mm	Slow growers	------	-------
10-14mm/48 hrs	Medium growers	FR 6,11, 14, 16, 26, 29, 30, 34, 38	FR 6, 7, 8, 9, 11, 14, 16, 20, 26, 27, 29, 30, 33, 34, 38
15-20mm/48 hrs	Fast growers	FR 1, 3, 4, 5, 7, 8, 9, 10, 12, 13, 15, 17, 18, 20, 21, 27, 31, 32, 33, 35, 36, 37	FR 1, 2, 3, 4, 5, 10, 12, 13, 15, 17, 18, 19, 21, 22, 23, 24, 25, 28, 31, 32, 35, 36, 37
21-25mm/ 48 hrs	Very fast growers	FR 2, 19, 22, 23, 24, 25, 28	-------

The effect of different cultural conditions on the growth and pigment production by *Fusarium moniliforme* causing bakanae disease has been reported by different workers (Pradeep *et al* 2013, Pannu *et al* 2013, Yadav *et al* 2014). From, the present findings conclusion can be drawn that cultural characteristics and the intensity of the pigment produced by *Fusarium moniliforme in vitro* are influenced by the nutrient medium. This proves the fungus produced colored variants that can be used for distinguishing among isolates. This phenotypical and their physiological study will help to understand the probable nature of pathogen growth under field conditions. This also showed the diversity in the nature of colony color and their growth.

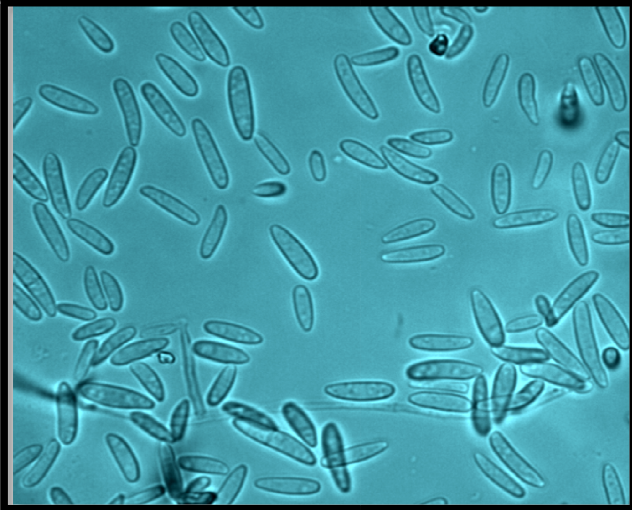
4.4 Morphological characterization of *F. moniliforme* culture

Obsevations on conidial shape, size, septation, formation of conidial chains and production of sporodochia were recorded with the help of compound microscope Leica DM 3000. Size of conidia was measured under the microscope using Image Analyzer Software at 40X. Microscopic observations showed that sporulation of micro-conidia formed on SNA media were high and the shape and size of the spore were more uniform and regular as compared to other media. This is supported by the fact that most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelial growth with ultimate loss of sporulation (UKNCC 1998). However, no macro-conidia were formed on the three media under study. As suggested by Leslie and Summerell (2006), CLA media was used for observations of macro-conidia. The detailed study has been described in Table 19. Production

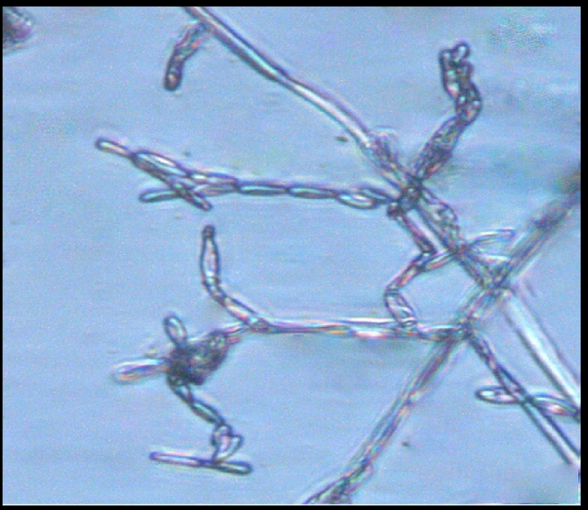
of micro and macro-conidia has been shown in Plate 5.

The size of the spores varied among the 38 isolates in the range of (6.8-9.8) X (2.9-6.0) µm for micro-conidia with no septa and (24.23-62.97) X (2.90-4.27) µm for macro-conidia with 3-5 septa. Micro-conidia were oval/ ovoid in shape with flattened base, usually 0-septate and produced in large numbers. Atukwase *et al* (2012) also reported globular or fusiform micro-conidia and were formed abundantly. Macro-conidia and sporodochia were produced on carnation leaf agar medium by all the isolates. Macro-conidia were septed, slightly curved and were canoe or needle-like appearance under the microscope with basal and apical cell. The basal cell appeared as distinctly notched or barely notched and apical cell as hooked. Ilija *et al* (2009) observed the macro-conidia with basal and apical cell which were described as delicate, slightly sickle-shaped or almost straight or narrow at both ends and occasionally somewhat bent into a hook at the apex and distinctly or slightly foot-celled at the base. Atukwase *et al* (2012) and Kaur *et al* (2014) also reported falcate to cylindrical macro-conidia with 3-5 septa. Yadav *et al* (2014) reported micro-conidial size of 5-12X 1.5-2.5µm with 0-1 septum and hyaline, long fusoid, tapered to the ends, straight or curved macroconidia of 25-60 X 2.5-4µm with 3-7 septa. The findings corroborate to the reports of Zainudin *et al* (2008a) and Bashyal *et al* (2015). Isolates varied in morphological characteristics but the shape and size of macro and micro-conidia were similar to those described by Leslie and Summerell (2006). Mycelium of *Fusarium moniliforme* is septate forming a dense coat of branching hyphae and had a width of 2-3µm. Microconidiophores are short, which may be mono or poly-phialides and simple arising laterally from hyphae and produce microconidia singly in chains (Ahangar *et al* 2014). Chains of micro-conidia were formed on water agar with KCl and chlamydospores were absent on all the isolates. Ilija *et al* (2009) reported that micro-conidia were more or less agglutinated in chains and remained joined or cut off in false heads. The results also agreed with various workers (Atukwase *et al* 2012, Kaur *et al* 2014, Ahangar *et al* 2014, Bashyal *et al* 2015). Based on the morphological and cultural characteristics studied, isolates were broadly categorized into five groups (Table 20). Group I comprised of white, milk white, antique white and pearl colored colony producing isolates with the size of micro-conidia ranging between (9.8-7.2)X(2.9-6.0) µm and macro-conidia size between (25.34-62.97)X (3.10-3.89) µm. However, FR19 has been put in Group I on the basis of colony color produced despite its macro conidial size (7.34 X3.68) µm differing from other members of the group. Group II consisted of rose, pink rose colored isolates with size of micro-conidia ranging from 6.8-8.9)X(3.5-4.9) µm and macro-conidia size of (28.17-32.85)X (2.91-4.08) µm. Group III consisted of dull purple and white colored isolates with size of micro-conidia ranging between (7.7-8.4)X(3.2-3.5)µm and macro-conidia size of (26.52-33.42)X (3.63-

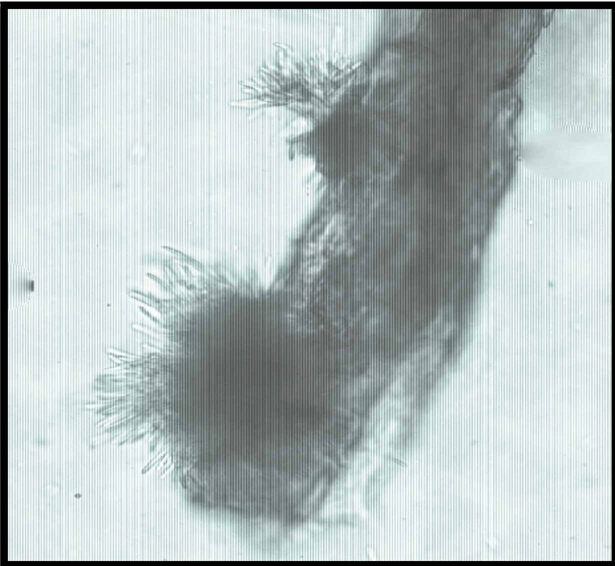
Plate 5. Different morphological features of *F. moniliforme* observed on different media



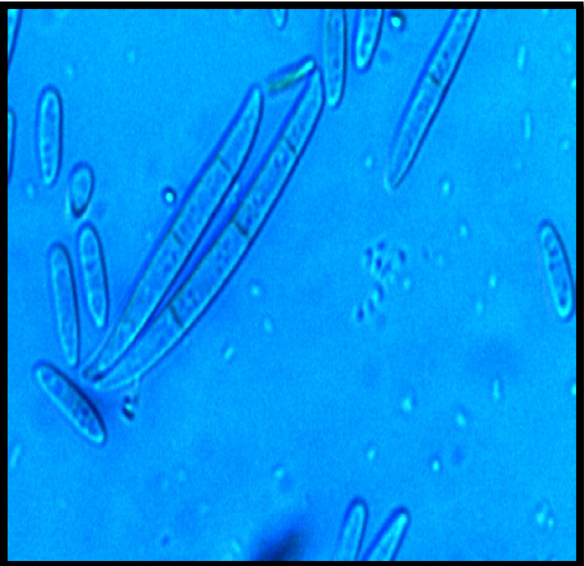
5.1 Micro-conidia on SNA media



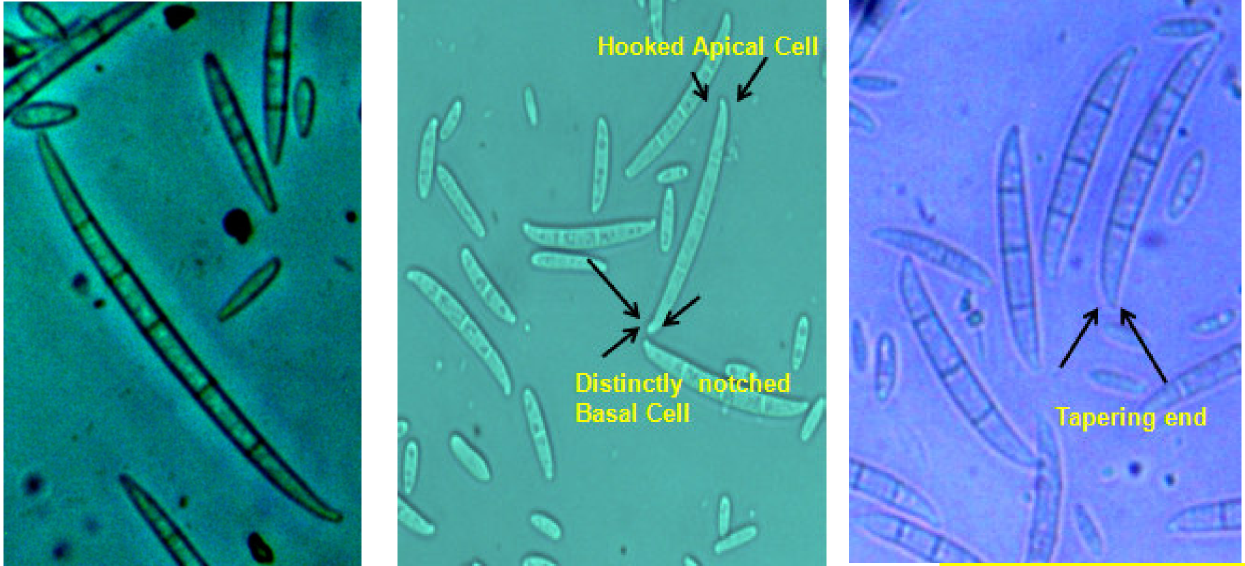
5.2 Micro-conidial chain on KCl media



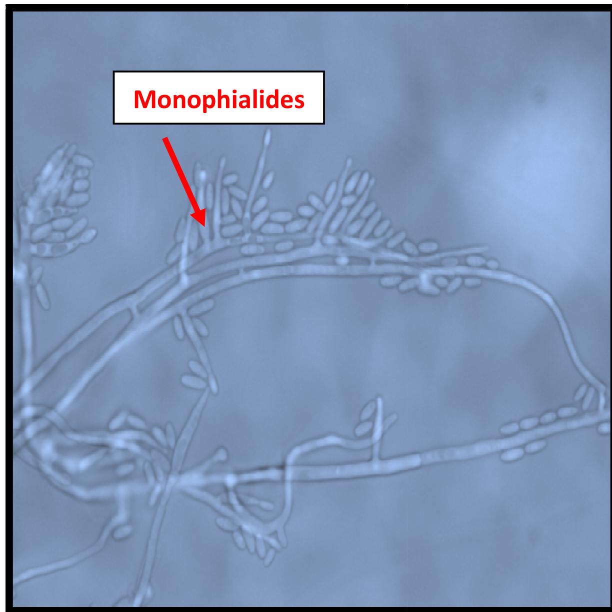
5.3 Sporodochia on CLA media

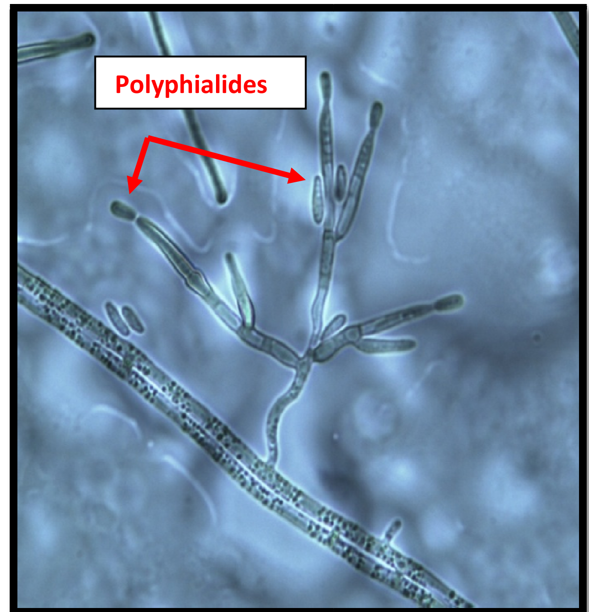


5.4 Macro-conidia on CLA media



5.5 Macro-conidia showing apical and basal cell





5.6. Monophialides and Polyphialides bearing micro-conidia

Table 19. Morphological characterization and variability of conidia of different isolates of *Fusarium moniliforme*

Morphological Characteristics		Isolates									
		FR1	**FR2**	**FR3**	**FR4**	**FR5**	**FR6**	**FR7**	**FR8**	**FR9**	**FR10**
Macro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope (µm)	Length (µm)	33.59	28.17	25.34	24.23	29.52	33.08	32.50	32.85	35.09	35.80
	Breadth (µm)	3.82	3.24	3.89	4.02	3.10	3.51	3.58	3.73	3.73	3.50
	Shape	*Typical	Typical	Typical	Typical	Typical	Typical	Typical	Typical	Typical	Typical
	Apical	Hook	Hook	Hook	Hook	Hook	Hook	Hook	Hook	Hook	Hook
	Basal	Distinctly notched	Distinctly notched	Barely notched	Barely notched	Barely notched	Distinctly notched	Barely notched	Distinctly notched	Distinctly notched	Barely notched
	Septa	3	3	3	3	3	3-4	4	3-4	3	3
Micro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope (µm)	Phialides	Poly	Mono	Poly	Poly	Mono	Mono	Mono	Poly	Mono	Poly
	Sporodochia	+	+	+	+	+	+	+	+	+	+
	Micro-conidial shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval
	Length	8.4	7.9	8.4	9.4	7.5	9.3	8.9	8.4	8.2	2.6
	Breadth	3.6	3.5	3.7	3.8	3.5	3.9	3.6	3.2	3.7	1.3

Table 19 contd..

Morphological characteristics		**Isolates**									
		FR11	**FR 12**	**FR 13**	**FR 14**	**FR 15**	**FR 16**	**FR 17**	**FR 18**	**FR 19**	**FR 20**
Macro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope (µm)	Length (µm)	32.71	30.99	32.49	46.14	29.00	33.42	28.24	35.94	7.34	45.17
	Breadth (µm)	3.73	3.49	3.74	3.48	3.63	3.85	3.35	4.33	3.68	4.42
	Shape	Typical	Typical	Typical	Typical	Typical (rarely form)	Barely notched	Typical	Dorsiventral curvature falcate	Typical	Typical
	Apical	Hook	Hook	Hook	Hook	Blunt	Hook	Hook	Hook	Hook	Blunt
	Basal	Distinctly notched	Barely notched	Barely notched	Distinctly notched	Barely notched	Distinctly notched	Barely notched	Distinctly notched	Barely notched	Barely notched
	Septa	4	3	3	3-4	3	3	3	4-5	3-4	3
Micro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope (µm)	Phialides	Poly	Mono	Mono	Poly	Poly	Poly	Mono	Mono	Mono	Poly
	Sporodochia	+	+	+	+	+	+	+	+	+	+
	Micro-conidial shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval
	Length	8.6	7.9	7.8	7.2	9.0	7.9	9.8	6.8	7.3	8.9
	Breadth	3..2	2.8	3.4	6.0	4.0	3.3	5.0	4.9	3.4	3.7

Table 19 contd..

Morphological characteristics		Isolates									
		FR 21	**FR 22**	**FR 23**	**FR 24**	**FR 25**	**FR 26**	**FR 27**	**FR 28**	**FR 29**	**FR 30**
Macro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope (µm)	Length	31.51	41.19	34.79	35.38	32.67	32.27	34.89	34.37	62.97	36.25
	Breadth	4.27	3.72	2.90	4.88	4.08	3.61	3.35	3.75	3.80	3.75
	Shape	Typical	Typical	Slender straight almost needle - like	Typical	Typical	Typical	Typical	Typical	Elongated slender	Typical
	Apical	Hook	Hook	Hook		Blunt	Hook	Hook	Hook	Hook	Hook
	Basal	Barely notched	Distinctly notched	Distinctly notched	Barely notched	Distinctly notched	Distinctly notched	Distinctly notched	Distinctly notched	Distinctly notched	Barely notched
	Septa	4-5	3	3	3	3	3	3	3	3-5	3
Micro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope (µm)	Phialides	mono	Mono/poly	Poly	Mono	Poly	Mono	Mono	Mono	Poly	Poly
	Sporodochia	+	+	+	+	+	+	+	+	+	+
	Micro-conidial shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval
	Length	8.9	7.7	7.7	8.4	7.9	8.4	9.4	7.5	9.3	8.9
	Breadth	3.5	2.9	3.4	3.6	3.5	3.7	3.8	3.5	3.9	3.6

Table 19 contd..

Morphological characteristics	Isolates								
Macro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope (µm)		**FR 31**	**FR 32**	**FR 33**	**FR 34**	**FR 35**	**FR 36**	**FR 37**	**FR 38**
	Length	31.32	25.29	30.83	31.41	33.03	32.38	26.52	31.41
	Breadth	3.49	3.36	2.91	3.45	4.19	4.15	4.08	3.45
	Shape	Typical*	Typical	Typical	Typical	Typical	Typical	Typical	Typical
	Apical	Hook	Hook	Hooked	Hook	Hook	Hook	Hook	Hook
	Basal	Barely notched	Barely notched	Barely notched	Barely notched	Barely notched	Distinctly notched	Barely notched	Barely notched
	Septa	3	3	3	3	3	3	3	3
	Phialides	Mono	Mono	Mono	Mono	Poly	Poly	Mono	Mono
	Sporodochia	+	+	+	+	+	+	+	+
Micro-conidial characterization of *Fusarium moniliforme* isolates using Leica microscope	Micro-conidial shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval
	Length	8.4	8.2	2.6	8.6	7.9	7.8	7.2	9.0
	Breadth	3.2	3.7	1.3	3.2	2.8	3.4	6.0	4.0

*Typical: long Slender falcate or straight

Table 20. Grouping of *F. moniliforme* isolates on the basis of cultural characteristics, micro-conidial and macro-conidial size

Category	Colony colour on SEA and PDA	Isolates	Micro-conidia size range (µm)	Macro-conidia size range (µm)	Chlamydospores	Sporodochia	Chains
GRP 1	White, Milk White, Antique White, Pearl	FR(1--6),(8-17), (19-20), 22, (24-25), (27-38)	(9.8-7.2)X(2.9-6.0)	(25.34-62.97)X (3.10-3.89)	absent	Present	Present
GRP 11	Rose, Pink Rose	FR 7, 18, 21, 26	(6.8-8.9)X(3.5-4.9)	(28.17-32.85)X (2.91-4.08)	absent	Present	Present
GRP 111	Dull purple and White	FR 5, 23, 31	(7.7-8.4)X(3.2-3.5)	(26.52-33.42)X (3.63-4.08)	absent	Present	Present
GRP IV	Peach and sea shell	FR 15, 17, 37	(9.09.8)X (4.0-5.0)	(24.23-35.94)X (3.36-4.27)	absent	Present	Present
GRP V	Velvet maroon and egg plant	FR 2, 8, 25, 33	(7.9-8.4)X(3.2-3.5)	(34.79-31.32)X (2.90-3.74)	absent	Present	Present

4.08)µm. Group IV consisted of peach and sea shell colored isolates having size of micro-conidia ranging from (9.0-9.8)X(4.0-5.0)µm and macro-conidia size (24.23-35.94)X (3.36-4.27) µm. The velvet maroon and egg plant colored colony with the size of micro-conidia ranging from (7.9-8.4)X(3.2-3.5) µm and macro-conidia size (34.79-31.32)X (2.90-3.74) µm kept in Group V. FR33 has been placed in Group V on the basis of colony color. However, its micro-conidial size is (2.6X1.3) µm different from the other members of the group. The categorization based on their morphological and cultural characteristics has also been described by Bashyal *et al* (2015). On the basis of the morphological data, it was confirmed from "The *Fusarium* laboratory Manual" by Leslie and Summerell (2006) that the isolates were identified as *F. moniliforme* (syn. *F. verticilloides*) or *F. proliferatum*. The further confirmation was performed using molecular techniques.

4.5 Molecular Characterization of *Fusarium* spp using Species specific primers

Identification based on cultural and morphological features only is not reliable, thus the identification of species requires confirmation using species specific primers. On the basis of morphological and cultural characters the thirty-eight isolates were identified as *F. moniliforme* (syn. *F. verticilloides*) or *F. proliferatum* (Leslie and Summerell 2006). The morphological identification was further confirmed by using species specific primers. DNA from 38 isolates was amplified with the set of primers VERT1 and VERT2 and 35 out of 38 isolates gave a single amplicon of 800bp (Plate 6). None of the isolates showed bands for the primers specific for *F. proliferatum*. VERT1 and VERT2 primers specific for *F. verticilloides*, based on intergeneric spacer region of Ribosomal DNA (Mirete *et al* 2004, Patino *et al* 2004, Kaur *et al* 2014) , were used because of their efficiency to discriminate among various species. Sreenivasa *et al* (2008) and Dissanayake *et al* (2009) also made the similar observations.

The cultural and morphological features indicated that the isolates belong to *Fusarium verticilloides* or *F. proliferatum*. However, molecular studies confirmed that the *Fusarium* spp. to be *F. moniliforme* (syn. *F. verticilloides*). These investigations revealed the diversity in the pathogen causing bakanae disease of Basmati in Punjab. The study confirms that identification based on cultural and morphological features only is not reliable, thus the identification of species using species specific primers are required for confirmation. From the growth rate of *Fusarium moniliforme* on three different media, it was also confirmed that PDA is the best media followed by SEA and SNA. PDA was triggered more intense production of pigment on culture as compared to other media. Studying of microscopic structures like macro-conidia has been challenging as the spores are not readily formed on most media even though these are abundantly available under natural conditions in the foot rot infected field. Carnation Leaf Agar media which supports productions of macro-conidia will contribute largely for identification of the species of different *Fusarium* which are

involved in causing foot rot of rice.

4.6 Pathogenicity isolates on susceptible cultivar Pusa1121

All the 38 isolates of *Fusarium moniliforme* (syn. *F. verticilloides*) were tested for pathogenicity by sowing artificially infected seed. Elongated and slender seedlings / bakanae symptoms appeared in the seedlings raised from artificially infected seed after 15 days of sowing. Mortality of the plants was observed within 30 days of inoculation. Diseased seedlings were pale yellow and often visibly taller than the healthy rice plants. Some of the seedlings remained stunted as compared to the normal ones. The few seedlings from the infected seeds died after few days of germination whereas other seedlings often progressively died from the seedling stage through to maturity. The findings agreed with the observation of Ilija *et al* (2009). Noticeable pink to white fungal growth and profuse sporulation of the fungus were observed on the lower portion of stems of diseased plants. On the basis of the symptoms they produced, all the 38 isolates were found to be pathogenic. The pathogen was re-isolated from the infected plants to prove the Koch's postulates. Number of stunted and elongated plants were recorded (Table 21) and disease incidence was calculated. All the isolates varied in terms of disease incidence and in the production of elongation and stunting symptoms. However, there is variation in the extent of pathogenicity or disease incidence on the susceptible cultivar Pusa1121. Similar observations on variability of pathogenicity has been observed by other researchers from different parts of the world (Zainudin *et al* 2008a, Amatullia *et al* 2010, Young-Ah *et al* 2013, Bashyal *et al* 2015). Some isolates also produced tanning and yellowing, confirming the observations of Zainudin *et al* (2008a) and Amataullia *et al* (2010). The isolate FR 1 scored the highest disease incidence (100 %) followed by FR 24 (96.67 %) and FR 18 (95 %). The isolate FR 12 has the lowest disease incidence (27.91%). Based on the pathogenicity, *Fusarium moniliforme* isolates were categorized as moderately virulent, virulent and highly virulent as depicted in Table 22. Single isolate FR 22 was moderately virulent, 11 isolates (FR 5, 10, 12, 16, 19, 25, 28, 29, 32,33, 37) were virulent and 26 isolates (FR1-4, 6-9, 11, 13-15, 17-18, 20-21, 23-24, 26-27, 30- 31, 34-36 and 38) were highly virulent (Table 22). Most of the isolates were highly virulent i.e., they produced disease incidence in the range of 51-100% followed by virulent (26-50%). Other researchers Sharma and Bagga (2007), Kaur *et al* (2009), Bashyal and Aggarwal (2013) also reported variation in pathogenicity of the *F. fujikuroi* and *F. moniliforme* based on mortality and symptoms produced by the pathogen including slender pale, elongation, stunting and overall incidence. Disease expression was found to be more or less dependent on the virulence of each isolate. Different levels of virulence have also been previously reported by Sunder and Satyavir (1998b). Such differences were aimed to characterize *Fusarium* spp. associated with plants providing data on *Fusarium* diversity and variability (Venturini *et al* 2013). Amatullia *et al* (2010) described different levels of aggressiveness and separated the various *Fusarium*

spp associated with bakanae disease of rice in Italy using EF-1α sequences. Many phenotypic markers, such as pathogenicity, growth rate, mycelia and conidial production may be used for describing the population biology of *F. moniliforme*. Among these, pathogenicity may constitute one of the most suitable characters for investigating fungal population variability (Zhan *et al* 2007, Haque *et al* 2008). This variation may be attributed to the genetic component of the isolates which further needs confirmation. Besides, variation in aggressiveness among the isolates may be due to physiological features of the isolates or by environmental factors, such as temperature and humidity, during the experiment itself as previously reported (Amataullia *et al* 2010).

Several researchers from different parts of the world have considered morphological and molecular characterization of *Fusarium verticilloides* based on their micro and macro-conidial features and pathogenic variability to study variation. Krnjaj *et al* (2007) reported high genetic diversity of *F. verticilloides* population based on pathogenicity and *nit1* and NitM and vegetative compatibility. Hirata *et al* (2001) from Japan described morphological and molecular characterization based on mitochondrial small subunit (mtSSU) rDNA and translation elongation factor (EF-1) introns and exons of *Fusarium verticilloides*. Gherbawy *et al* (2002) identified *Gibberella fujikuroi* complex from Austria using RAPD and they indicated that the *Fusaria* species were distinct species based on their amplification patterns. Rahjoo *et al* (2008) also identified the *Fusarium* isolates both morphologically and using species specific primers VER1/2, PRO1/2 and translation elongation factor 1-α (TEF) gene region. Zainudin *et al* (2008a) reported bakanae disease of rice from Malaysia and Indonesia and described etiology of the disease based on morphological, physiological and pathogenic characteristics. They also categorized the isolates based on their pathogenicity as pathogenic, slightly and not pathogenic. Hsuan *et al* (2010) findings also proved that morphological characterization is complemented by molecular technique (RFLP-IGS analysis). They determined genetic relationships between the *Fusarium* species isolated from rice, sugarcane and maize and clustered these based on similarity value. Mohammadian *et al* (2011) also reported genetic diversity of *Gibberella moniliformis* and *G. intermedia* from corn and rice.

Table 21: Variability in the symptoms and disease incidence of foot rot.

Isolates I.D.no.	Stunting (%)	Elongation (%)	Disease incidence (%)
FR1	8.00	92.00	100.00^{a}
FR2	20.00	45.00	65.00^{g}
FR3	12.5	67.50	80.00^{e}
FR4	23.40	34.04	57.45^{ijk}
FR5	9.09	34.09	43.18^{op}
FR6	75.00	12.50	87.50^{d}
FR7	25.00	29.55	54.55^{jkl}
FR8	43.18	13.64	56.82^{ijk}
FR9	8.57	57.14	65.7^{1fg}
FR10	8.33	27.78	36.11^{q}
FR11	0.00	65.79	65.79^{fg}
FR12	4.65	23.26	27.91^{r}
FR13	6.67	53.33	60.00^{hi}
FR14	40.00	40.00	80.00^{e}
FR15	44.44	11.11	55.56^{ijk}
FR16	32.61	17.39	50.00^{lmn}
FR17	12.77	65.96	78.72^{e}
FR18	13.89	91.67	95.00^{b}
FR19	20.83	27.08	47.92^{n}
FR20	8.69	71.74	80.43^{e}
FR21	23.68	39.47	63.16^{gh}
FR22	8.89	15.56	24.44^{r}
FR23	13.51	67.58	81.08^{e}
FR24	23.33	73.33	96.67^{bc}
FR25	30.00	20.00	50.00^{lmn}
FR26	15.22	43.48	58.70^{hij}
FR27	18.92	62.16	81.08^{e}
FR28	20.00	28.89	48.89^{mn}
FR29	26.32	21.05	47.37^{no}
FR30	27.03	27.03	54.05^{jkl}
FR31	42.55	12.77	55.32^{jkl}
FR32	6.45	32.26	38.71^{pq}
FR33	20.00	20.00	40.00^{pq}
FR34	65.79	13.16	78.95^{e}
FR35	13.16	71.05	84.21^{de}
FR36	10.00	60.00	70.00^{f}
FR37	15.38	30.77	46.15^{no}
FR38	35.00	17.50	52.50^{klm}

*Data followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 22: Grouping of *F. moniliforme* isolates on the basis of pathogenicity

Sl.no.	Disease incidence	Virulence rating	No.	Isolates
1	2-25%	Moderately virulent	1	FR 22
2	26-50%	Virulent	11	FR 5, 10, 12, 16, 19,25, (28-29), (32-33), 37
3	51-100%	Highly virulent	26	FR (1-4),(6-9),11,(13-15), 17, 18, 20, 21, (23- 24), (26-27), (30-31), (34-36), 38

*The scale has been adopted from IRRI Standard Evaluation System for Rice

Bahmani *et al* (2012) also investigated genetic diversity based on vegetative compatibility in Iran using RAPD and reported high polymorphism.Young-Ah *et al* (2013) described the incidence, molecular characterization based on tubulin and pathogenicity of GFSC complex associated with rice seeds from Asian countries. From all the findings and reports from the present work and from different parts of the world, conclusion can be made that *Fusarium* spp associated with Bakanae are highly variable based on morphological, pathological and molecular diversity. It was also observed that the variability study among the isolates is not complete with only morphological characterization. To complement the finding, molecular characterization using different types of markers is needed. Besides, identification based on morphological is not reliable as visual observation may sometimes be confusing in determining the closely related species like *F. moniliforme* and *F. proliferatum*. So, to identify the species accurately, molecular technique using different species specific primers is a must. From the findings, it is revealed that under Punjab conditions, *F. verticilloides* (syn *F. moniliforme*) is responsible for causing bakanae disease in Basmati.The morphological studies, molecular characterization and pathogenicity studies revealed that variable population exists in nature in different parts of Punjab.

4.7 Characterization of Bio-chemical variability

Fusaric and gibberellic acid are the important secondary metabolites produced by *Fusarium* spp. The presence of the fusaric acid (FA) and gibberellic acid (GA) produced in culture was detected using TLC plate (Plate7). All the 38 isolates produced GA; however, 30 isolates produced FA (Table 23). The isolates that showed positive results for the production of FA qualitatively on TLC plate were determined for the quantitative production of FA spectrophotometrically. The quantity of FA was estimated from the standard curve generated using sigma standard (Fig. 1). Fusaric acid was produced at the range of 0.80-6.40 µg/ml. Only the isolate FR 1 produced FA concentration below 1 µg/ml. It produced the lowest concentration of FA among the 30 isolates whereas FR 6 produced the highest FA. On the basis of quantitative estimation of FA, the isolates can be categorized as not produced, low producers (<1 µg/ml), moderate producers (1-3 µg/ml) and high producers (>3 µg/ml) (Table 24). Eight isolates (FR 5, 9, 10, 11, 12, 13, 32, 35) failed to produce FA, single isolate FR1 produced <1 µg/ml, 19 isolates (FR 2, 3, 7, 14, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28,

Plate 6. Molecular confirmation of the species using VERT1 and VERT2 primers showing amplification at 800 bp

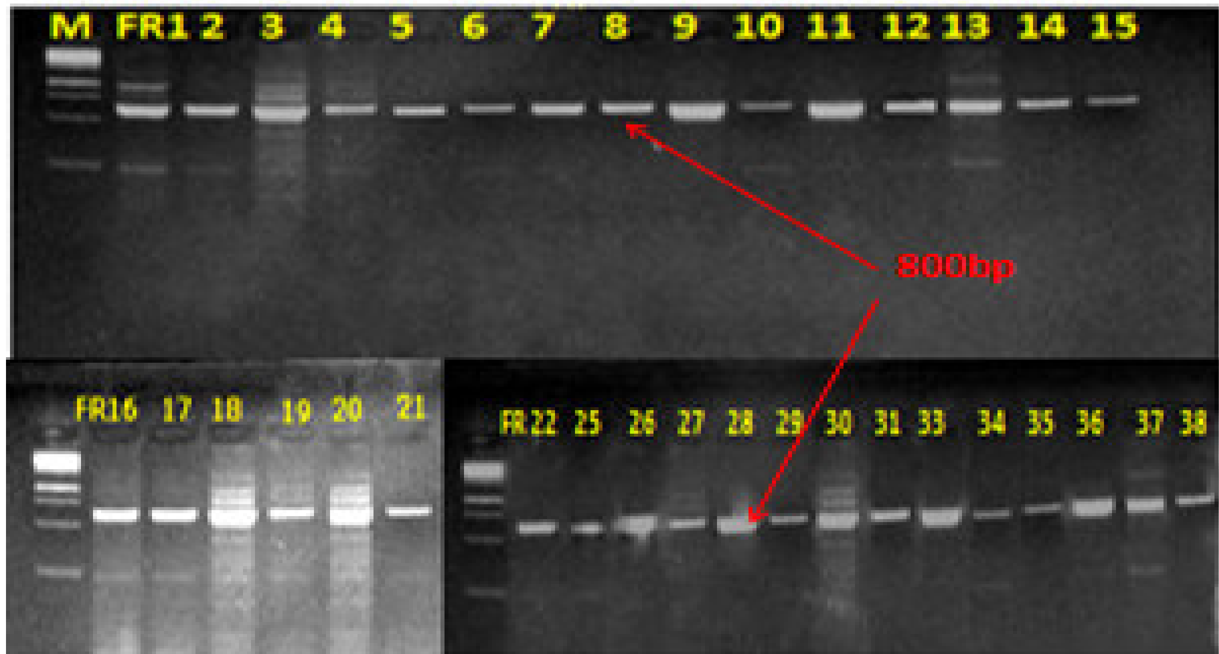


Plate 7. Pinkish bands produced on TLC plate showing the presence of fusaric acid in the sample

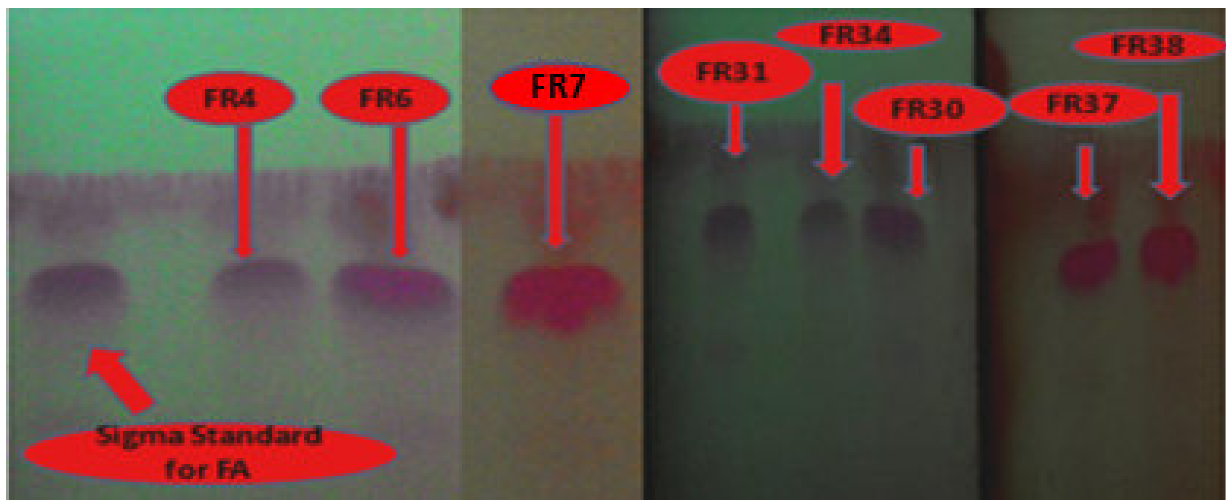


Table 23. Comparative production of gibberellic Acid and fusaric Acid by *Fusarium moniliforme* isolates in culture under *in vitro* conditions

Isolates	FA (µg/ml)	GA (µg /ml)	Isolates	FA (µg/ml)	GA (µg /ml)
FR 1	0.8[p]	23.4[a]	FR 20	1.23[o]	23.00[b]
FR 2	2.06[jk]	15.00[h]	FR 21	2.27[j]	14.87[h]
FR 3	1.67[l]	20.07[d]	FR 22	1.83[l]	9.23[l]
FR 4	3.63[g]	12.40[j]	FR23	1.73[l]	19.20[e]
FR 5	0.00[q]	14.83[h]	FR24	2.05[j]	17.37[g]
FR 6	6.40[a]	3.23[o]	FR25	3.83[f]	9.83[k]
FR 7	1.57[mn]	14.67[ih]	FR26	2.07[jk]	17.57[g]
FR 8	4.57[d]	9.07[l]	FR27	2.03 [jk]	18.17[f]
FR 9	0.00[q]	17.67[g]	FR 28	2.23[j]	14.83[h]
FR 10	0.00[q]	14.57[ih]	FR 29	3.30[h]	12.43[j]
FR 11	0.00[q]	19.17[e]	FR 30	3.20[h]	12.63[j]
FR12	0.00[q]	14.67[ih]	FR31	5.00[c]	6.00[m]
FR13	0.00[q]	18.27[f]	FR32	0.00[q]	14.23[i]
FR14	3.07[h]	14.67[ih]	FR33	3.05[h]	14.23[i]
FR15	5.12[c]	4.07[n]	FR34	5.40[b]	4.00[n]
FR16	4.30[e]	9.17[l]	FR35	0.00[q]	20.03[d]
FR 17	1.30[o]	21.17[c]	FR36	1.37[no]	18.37[f]
FR 18	1.30[o]	21.37[c]	FR37	1.40[n]	15.00[h]
FR19	2.57[i]	14.63[ih]	FR38	3.90[f]	9.43[lk]

*Data followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.
*FA- Fusaric Acid
*GA- Gibberellic Acid

29,30, 36, 37) were termed as moderate producers and 10 isolates (FR 4, 6, 8, 15, 16, 25, 31, 33, 34, 38) as high producers. Quantitative estimation of GA in the samples was determined spectrophotometrically and estimation of the concentration of GA was found out from the standard curved (Fig. 2). All the 38 isolates showed positive results for the production of GA by TLC. There was a single spot present in each of the test isolate and retention factor (*Rf*) of the GA spot in test isolate corresponded that of the standard GA (*Rf* =0.6). All the isolates showed variable production of GA (Table 23). The isolates produced GA in the range of 3.23-23.4 µg/ ml. On the basis of gibberellin production, fungal isolates were classified as low producer (<10 µg/ ml), moderate producer (11-20 µg/ ml) and high producer (>20 µg/ ml) (Table 25).

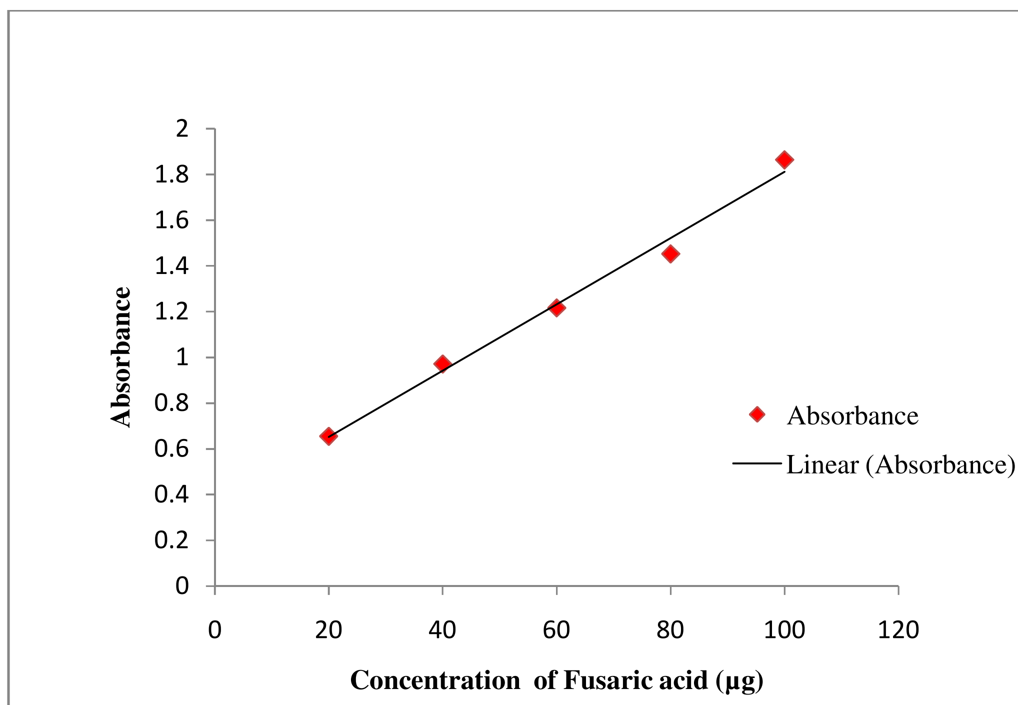


Fig. 1 Standard curve showing Absorbance against concentration of Fusaric acid

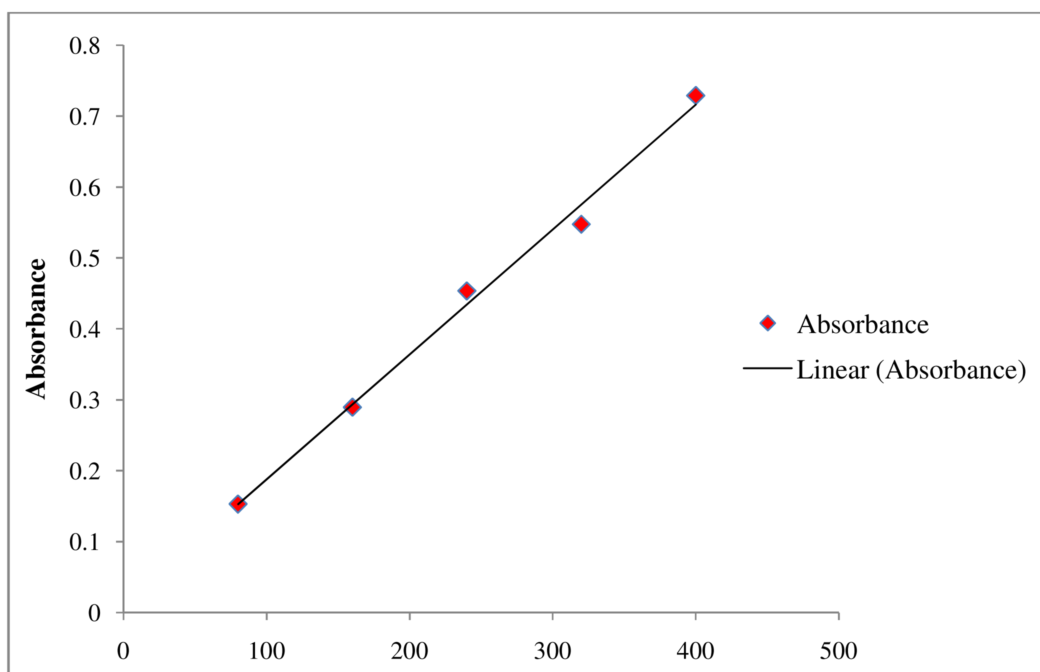


Fig. 2 Standard curve showing Absorbance against concentration of Gibberellic acid

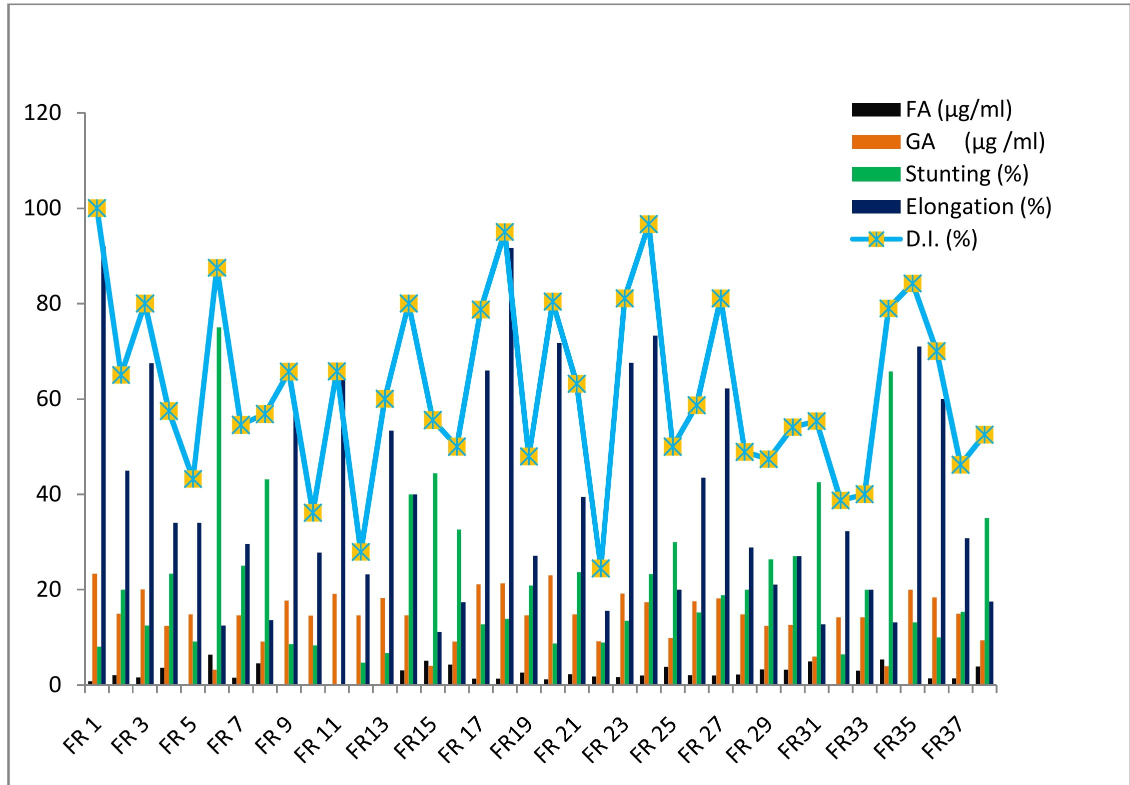


Fig. 3 Comparative production of gibberellic Acid and fusaric Acid by *Fusarium moniliforme* isolates and symptom produced on rice seedlings

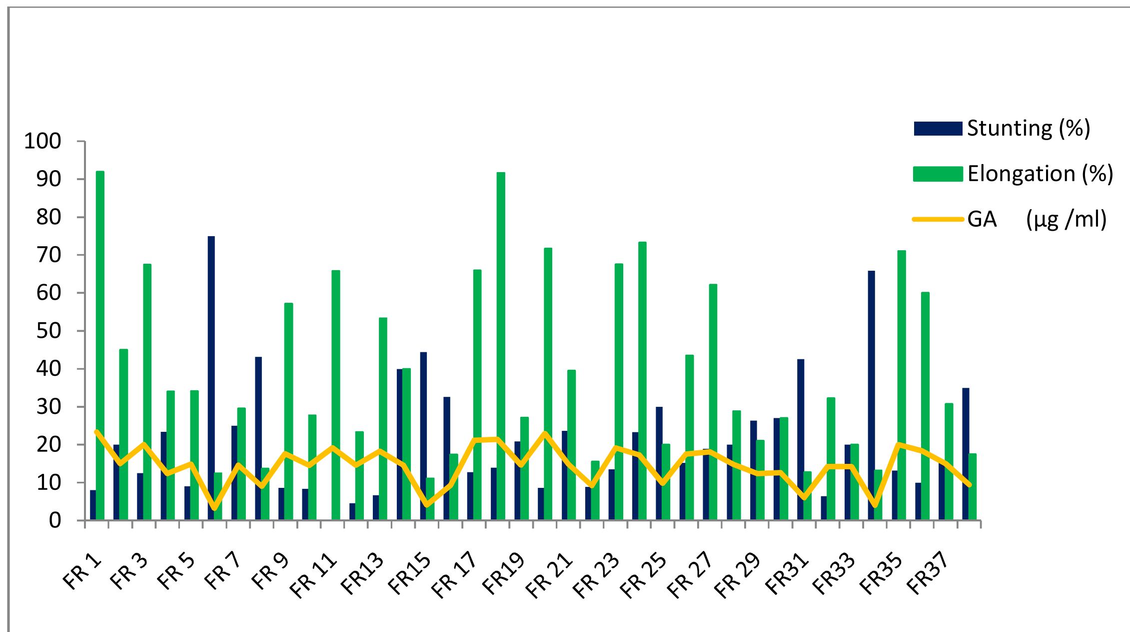


Fig. 4 Effect of Fusaric Acid produced by *Fusarium moniliforme* isolates on production symptoms

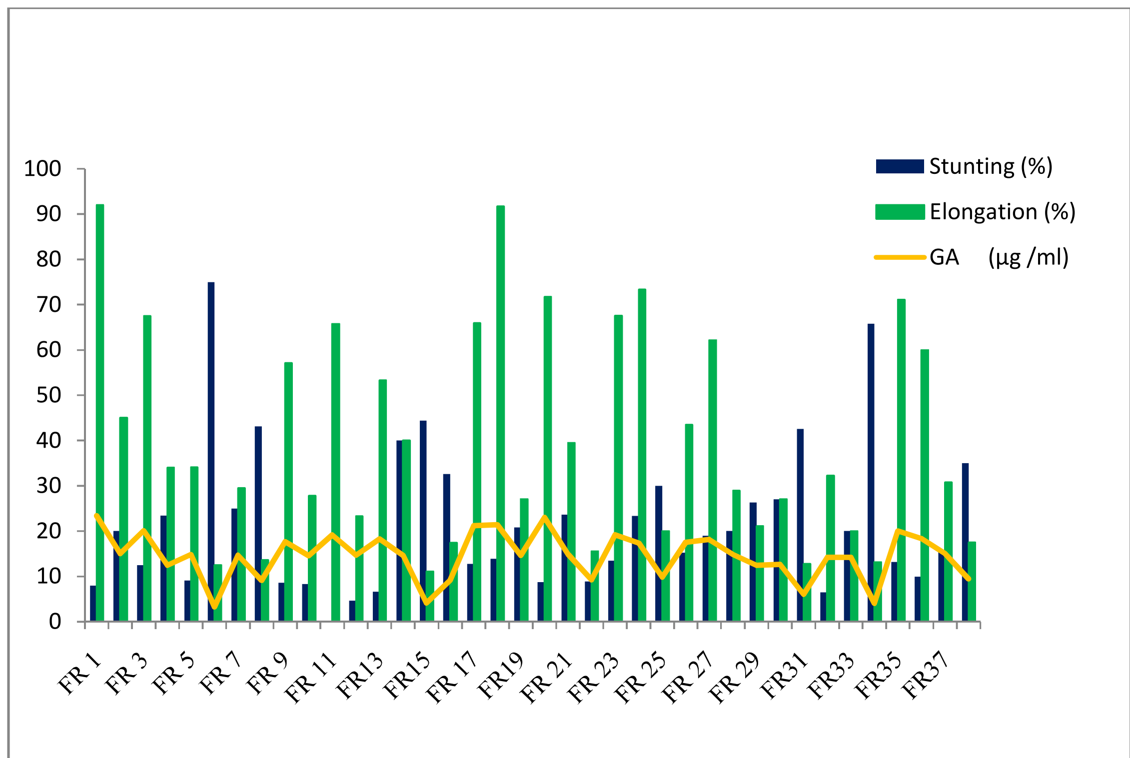


Fig. 5 Effect of Gibberellic Acid produced by *Fusarium moniliforme* isolates on production of symptoms

Table 24. Categorization of *F. moniliforme* isolates on the basis of fusaric acid production

Sl no.	Category	Range	No.	Isolates
1	None producers	0 µg/ml	8	FR 5, (9-13), 32, 35
2	Low producers	<1 µg/ml	1	FR 1
3	Moderate producers	1-3 µg/ml	19	FR (2-3), 7, 14, (17-24), (26-30), (36-37)
4	High producers	>3 µg/ml	10	FR 4, 6, 8, (15-16), 25, 31, (33- 34), 38

Nine isolates [6, 8, (15-16), 22, 25, 31, 34, 38] were low producers, 25 isolates [FR (2-5), 7, (9 -14), 19, 21, (23-24), (26-30), (32-33), (35-37)] were moderate producers and four isolates [FR 1, (17-18), 20] were high producers. A close analysis showed that most of the isolates produced 10-20µg/ml GA and therefore, most of the isolates belong to moderate producers. Among the high GA producers, FR 1 (23.4 µg/ ml) is the highest followed by FR 20 (23 µg/ ml), FR 17 and FR 18. FR 6 is the lowest GA producer.

Table 25. Categorization of *F. moniliforme* isolates on the basis of gibberellic acid production

Sl. No.	Category	Range	No. of isolates	Isolates
1	Low producer	<9 µg/ml	9	FR 6, 8, (15-16), 22, 25, 31, 34, 38
2	Moderate producer	>9µg/ml	25	FR (2-5),7, (9 -14), 19, 21, (23-24), (26-30), (32-33), (35-37)
3	High producer	>20 µg/ml	4	FR 1, (17-18), 20

All the isolates showed variable production of GA and FA. Fusaric acid was produced by 30 isolates out of 38 and GA has been produced by all 38 isolates that showed the maximum involvement of GA in malfunctioning caused by the disease. The results corroborates to the findings of Kaur *et al* (2011) and Kaur *et al* (2014) who also reported that *Fusarium* spp causing bakanae had the ability to produce GA whereas not all the isolates produced FA. Bhalla *et al* (2010) also reported that 28 isolates of *Fusarium* spp produced variable GA and were categorized as low, moderate, and high gibberellin producing strain. Lale *et al* (2006) reported *Gibberella fujikuroi* (teleomorph) produced variable GA and FA estimated by high performance liquid chromatography. Bacon *et al* (1996) assayed 78 different isolates of *Fusarium* spp. and reported that all the cultures tested positive for the

production of fusaric acid. Variability observed for GA production by the different strains of *Fusarium moniliforme* may be due to the different metabolic pathways of gibberellin productions (Bhalla *et al* 2010). It was established that GA12-7-aldehyde is a branch point to the various GAs and several pathways have been established from this branch point (Bearder *et al* 1975, Tudzynski *et al* 1999). Differences mainly in the position and sequence of hydroxylation and more than one pathway from GA12-7-aldehyde may be the reason for variation in gibberellin production by different fungal strains. Other reason for variations may be the differences in the geographical regions (Rojas *et al* 2001). Recently, Niehaus *et al* (2014) identified and characterized the fusaric acid gene cluster in *F. fujikuroi* consisting of the PKS-encoding core gene and four co-regulated genes, FUB1–FUB5. They reported FUB1 and FUB4 were necessary for the biosynthesis of the secondary metabolites. So, the isolates which failed to produce FA may be attributed to the presence and absence of fusaric acid gene cluster but this needs further investigations.

4.7.1 Correlation of GA and FA production by *F. moniliforme* with pathogenicity

Different isolates showed different levels of virulence that characterizes *Fusarium* diversity and variability (Venturini *et al* 2013).Variability in the production of GA and FA by all the isolates and their ability for causing bakanae in rice are complied with the findings of other researchers (Bacon *et al* 1996, Lale *et al* 2006, Bhalla *et al* 2010, Kaur *et al* 2011, Kaur *et al* 2014). The findings reveal the maximum involvement of GA in the production of the disease symptoms i.e., role of GA in development of the disease is more significant than FA. The level of production of GA and FA were observed to be correlated to the number of elongated and stunted plants, respectively. Statistical analysis showed that the production of FA significantly correlates positively with the number of stunted plants in pathogenicity test and GA production positively correlated with the number of elongated plants. Positive correlation between GA production and elongation symptoms (0.889) confirmed the role of GA causing foolish seedling type symptoms (Table 26). The result corroborates the finding of Sunder and Satyavir (1998b) and Ma *et al* (2008). Similarly, FA production and the number of stunted plants showed positive correlation (0.917) and this indicated the involvement of fusaric acid in causing stunting type of symptoms in foot rot infected plants. Some isolates (FR 5, FR 9, FR 10, FR 12 and FR 13) didn't produce FA. However, all of them produced the stunting type of symptom. This can be explained by the findings of Niehaus *et al* (2014). They identified and characterized the FA gene cluster in *F. fujikuroi* and also reported higher expression of this gene cluster in its favourite host plant rice compared to maize. This implies level of expression of gene responsible for FA production depends on the host. Since, the present study is based on the in *vitro* production of FA and cultures were grown in artificial medium and there is no interaction with the host, some isolates may have failed to express the gene under *in vitro* conditions. However, they have expressed the symptoms when tested for

pathogenicity. Positive correlation co-efficient of GA with disease incidence (0.403) and FA with disease incidence (0.041) indicated that both GA and FA play an essential role in the development of the foot rot disease (Table 23 and 26). This has also been evident from the work of other researchers (Nyvall 1999, Abo-Elanga and Ahmed 2007). From Table 23 and 26, the results showed that disease incidence is a cumulative effect of elongation and stunting symptoms inspite of FA and GA being negatively correlated. However, the case is not always true as some isolates have failed to produce FA. The case is feasible only if the isolates produced both GA and FA. But for those that produce either GA or FA, D.I is the percent diseased plants that showed either elongation or stunting (as per the virulence factors they produced). This is explained by the fact that disease incidence is measured on the basis of visible elongation and stunted symptoms per total number of plants observed (Zainudin *et al* 2008a). Thus, it can be concluded that the level of GA and FA affect the disease incidence individually as well as a unison factor in the production of the disease. Presence of both the factors or of either of them results in disease incidence. Comparing the contribution of GA and FA in the disease incidence, it was observed that the role of GA is more profound. The comparative studies have been indicated in fig. 3, 4 and 5.

Table 26. Correlation co-efficient between gibberellic and fusaric acid produced by different isolates and the symptoms produced

Correlation co-efficient	Gibberellic acid	Fusaric acid	Elongation	Stunting	Disease incidence
Gibberellic acid	-	-0.794*	0.889**	-0.800*	0.403^{ns}
Fusaric acid	-0.794*	-	-0.602^{ns}	0.917**	0.041^{ns}

* Significant (P<0.05); ** Highly significant (P<0.01); ns: Non-significant

The perusal of all these findings revealed that there is variation in pathogenicity and production of two important virulence factors: GA and FA by *F. moniliforme* pathogen. The role of GA and FA in pathogenicity cannot be ruled out in the development of the bakanae disease. The secondary metabolites GA and FA are responsible for the development of the disease and also the factor of pathogenic variability of the pathogen. This indicated the correlation between the pathogenic variability of the isolates with the biochemical variability. Understanding the pathogenic and bio-chemical variability of the isolates will provide an insight about the potential of the pathogenic isolates in development of the disease. This relationship of pathogenicity will help in the development of the suitable management strategies for foot rot.

4.7.2 Cluster analysis on the basis of bio-chemical properties

On the basis of the data of GA and FA production and disease incidence, cluster analysis was performed using SPSS software (SPSS Inc., version 17.0) after transforming the variables into Z-scores so that all the variables have equivalent weightage. All the isolates of

F. moniliforme were grouped into two main clusters: cluster I (moderate to high producers of GA and low to moderate producers of FA) and cluster II (high producers of FA and low GA producers).

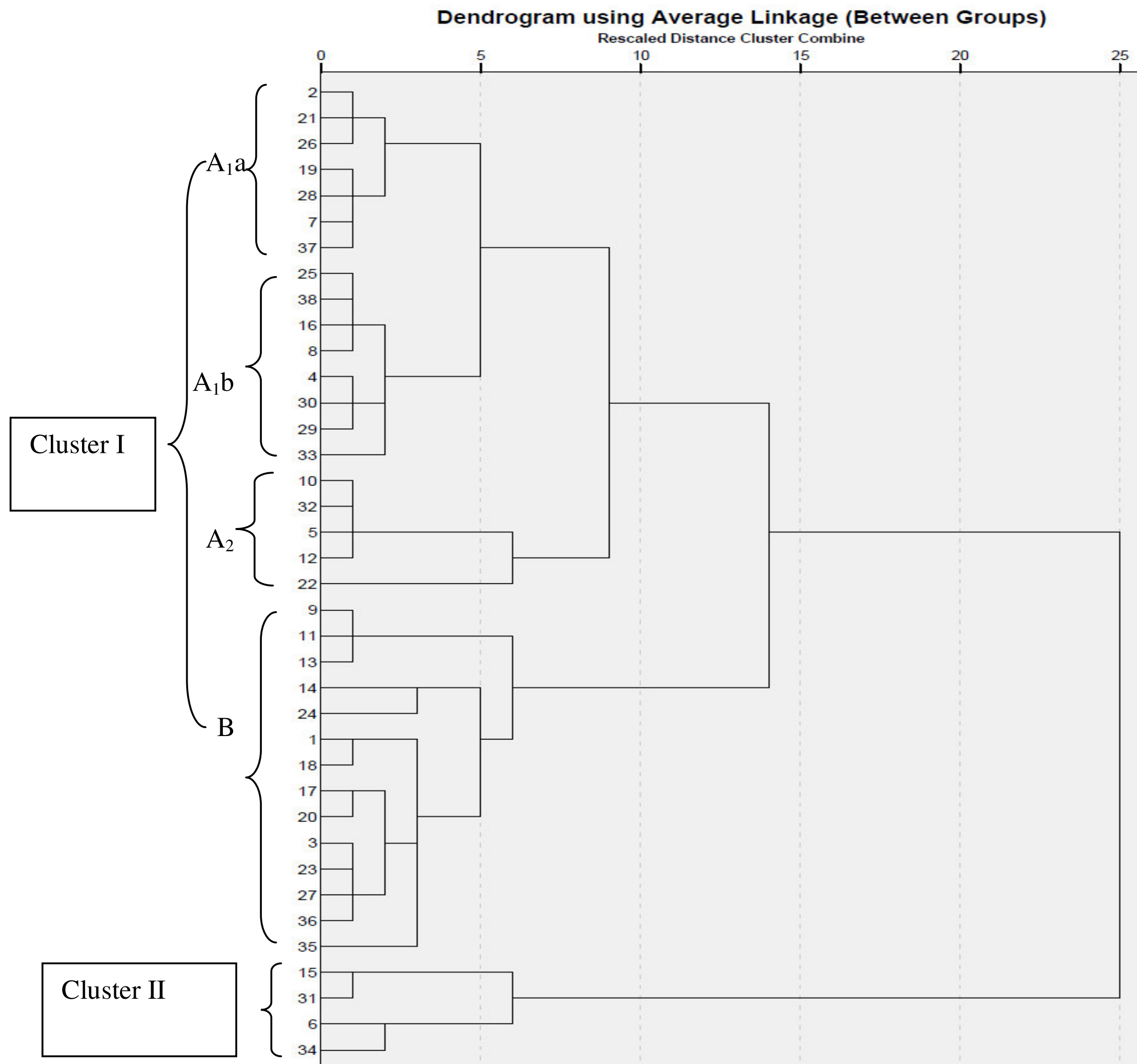


Fig.6 Dendrogram generated from quantitative data of Gibberellic, Fusaric acid and pathogenicity (z-scores).

Within cluster I, the isolates (FR 2, 21, 26, 19, 28, 7, 37) were sub-clustered as A_1a (moderate producers of GA and FA) and the isolates (FR 25, 38, 16, 8, 4, 30, 29, 33) as A_1b (moderate GA and high to moderate FA). The isolates (FR 10, 32, 5, 12, 22) were clustered as A_2 (isolates that didn't produce FA clubbed with moderate GA producers). The other sub-cluster B consists of the isolates (FR 9, 11, 13, 14, 24, 1, 18, 17, 20, 3, 23, 27, 35 and 36) that produced moderate to high GA along with moderate FA producers and those which doesn't produced FA. Cluster II consists of only four isolates (FR 15, 31, 6, 34) that produced high FA and low GA.

The finding clearly revealed the existence of variation in pathogenicity and production of GA and FA by different isolates *F. moniliforme*. The pathogenic variability may be due to the pooled effect of genetic, physiological and geographical differences and the biochemical variability may be attributed to the alteration in the biosynthetic molecules of GA (Bearder *et al* 1975, Tudzynski *et al* 1999) whereas the presence/ absence of FUB gene cluster responsible for FA production (Niehaus *et al* 2014). These secondary metabolites play significant role in pathogenicity of *F. moniliforme*. Thus, the role of GA and FA in pathogenicity cannot be ruled out in the bakanae disease. This has been attributed to the fact that fusaric acid and gibberellic acid produced by *F. moniliforme* (syn. *F. verticillioides*) caused stunting and elongation, respectively in many species of plants besides rice (Sood 1964, Young-Ah *et al* 2013). The production of FA and GA differs with the strain of the fungus which explained the variation of symptoms (Zainudin *et al* 2008b).

4.8 Molecular diversity analysis of *Fusarium moniliforme* using SSR markers

In the present study, 50 primers were selected from the sequence given in the paper authored by Xu *et al* (2012) to assess the genetic diversity among the 38 isolates of *Fusarium moniliforme.* Among the 50 primer pairs designed, 27 pairs (54%) were able to amplify 1or more alleles against discretionary *F. verticillioides* (Table 27). Eleven primers (1H02, 2H17, 2H15, 3H02, 3H19, 5H09, 9H05, 10H09, 10H01 and 11H01) amplified more than two alleles. A total of 102 alleles were detected by 27 primers from the 38 isolates of *Fusarium moniliforme*. An average of 2.74 alleles was produced per primer. Eleven primers (40.7%) produced more number of alleles than the average value. Out of 27 markers, all of the markers showed polymorphism with least polymorphism shown by primer (8H01) and no polymorphism by primer 2H06. The number of alleles generated by each primer varied from 1 to 10. Maximum number of alleles was produced by the primer 5H09. Amplification results by some selected primers are given (Plate 8). Variable allelic diversity using SSR markers have been reported by other scientists (Ravi *et al* 2003, Xu *et al* 2012).

Number of polymorphic bands was calculated from the DNA data matrix. A total of 102 molecular bands were produced and out of these 100 were polymorphic bands. Highest number of polymorphic bands was produced by primer 5H09 and 3H19 i.e., 13 polymorphic bands, followed by 11H01 and 1H02 i.e., 12 and 11 polymorphic bands respectively.

Polymorphic Information Content (PIC) is the reflection of allelic diversity and frequency among the isolates and varied greatly for all SSR loci tested. The PIC values varied from 0.10-0.89 with an average of 0.46 (Table 27). 11 SSR primers revealed PIC values higher than the average.

Among these; 7H05, 11H01, 5H09, 3H19, 10H09, 10H01, 9H05, 1H02, 2H17, 2H15, 3H02, 11H03 and 10H07 showed PIC values equal to or higher than 0.50. These markers are highly informative as they indicated high polymorphism. The PIC value can be looked as the

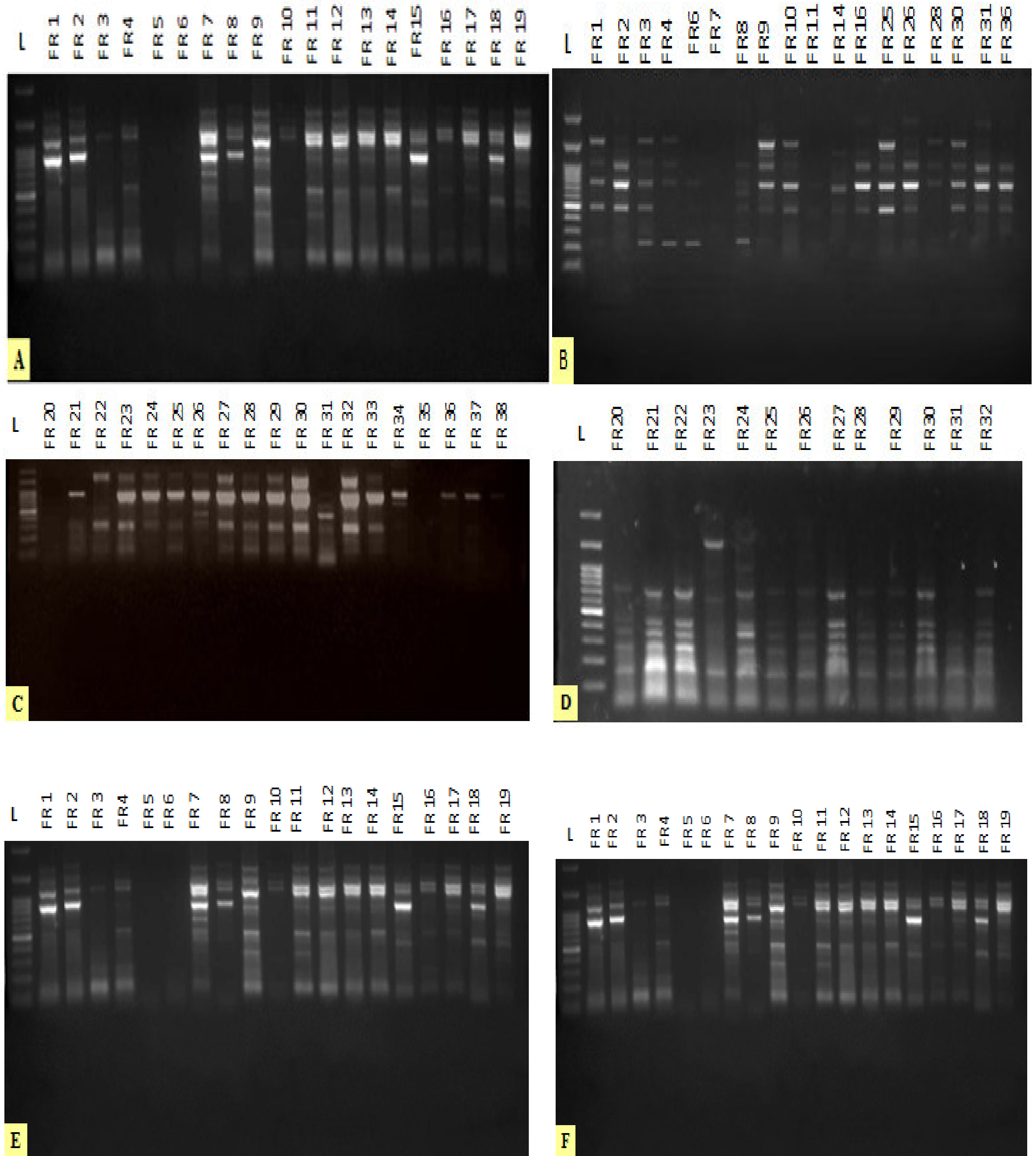
measurement of usefulness of each primer in distinguishing one individual from another (Bousba *et al* 2012). DeWoody *et al* (1995) emphasized that markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of marker at specific locus.

Table 27: Primers with their Polymorphic Information Content (PIC) values

Sl.no.	SSR primers	Total number of amplified fragments	No. of polymorphic bands	PIC value
1	1H01	1	1	0.42
2	1H02	11	11	0.88
3	2H13	1	1	0.11
4	2H17	5	5	0.78
5	2H15	4	3	0.50
6	2H05	1	1	0.15
7	2H14	1	1	0.46
8	2H06	1	1	0.29
9	3H02	6	6	0.82
10	3H07	1	1	0.38
11	3H09	1	1	0.38
12	3H19	13	13	0.89
13	4H01	1	1	0.29
14	5H04	1	1	0.42
15	5H06	1	1	0.46
16	5H10	1	1	0.33
17	5H09	13	13	0.89
18	7H05	6	5	0.57
19	7HI6	1	1	0.15
20	8H01	1	1	0.10
21	9H05	3	3	0.61
22	10H07	1	1	0.50
23	10H05	1	1	0.20
24	10H09	8	8	0.78
25	10H01	5	5	0.73
26	11H03	1	1	0.63
27	11H01	12	12	0.87
Total no. of bands		102	100	

Genetic dissimilarity was calculated from the matrix of binary data using software DARwin 6.0 where ‘0’ and ‘1’ were standardized as the least and maximum of dissimilarity respectively. To ascertain the statistical strength of genetic relationships identified through this analysis, bootstrapping of the data (1000 permutations) was performed. The dissimilarity coefficients were used for cluster analysis based on unweighted neighbor joining method and

Plate 8 PCR amplification products of *Fusarium moniliforme* isolates obtained by using Simple Sequence Repeats primers (A: 5H09; B: 11H01; C:3H19; D: 10H09, E: 5H09; F:1H02)



a dendrogram was generated with the aim of analyzing the relationships between 38 *Fusarium moniliforme* isolates studied. The genetic dissimilarity index ranged from 25 to 75%. The dendrogram indicated that the 38 isolates were clustered into two distinct clusters (Table 28). Cluster I is smaller than Cluster II consisting of five isolates (FR20, FR 18, FR 38, FR19 and FR16). Cluster II is the largest and comprised of 33 isolates, constituting of two sub groups: IIA and IIB. Subcluster IIA constitutes three isolates and it is smaller than the subcluster IIB. Subcluster IIB consists of another three subclusters $IIB_{1,}$ IIB_2 constituting of seven isolates each and IIB_3 with sixteen isolates.

The study revealed that morphological grouping, pathogenicity data and bio-chemical data showed some resemblance to clustering based on molecular data. However, the individual group based on phenotypic and bio-chemical did not locate distinctly in a particular cluster. Majority of the isolates of each group locate to a particular cluster but not all. A few of them were found to locate in another group. GRP1 was scattered all over the clusters. This can be explained by the fact that GRP1 constitutes those morphological and cultural characteristics that are commonly observed in all the isolates. The GRP II isolates FR 7, FR 21and FR 26 lie on cluster II B_3 but FR 18 locate in cluster I. Same case has been observed for GRIII where the isolates FR 23, FR 31 lie in cluster IIB_3 and FR in cluster IIB_1. For GRPIV, except for FR 37 in cluster IIA, other members FR 17, FR 15 lie in cluster IIB_2. Considering GRPV, FR 2 and FR 8 lie in cluster IIB1 and FR 25 and FR 33 lie in Cluster IIB_3.

The cluster generated based on the molecular data was also compared with the pathogenicity data and it was observed that disease incidence of the isolates present in cluster I varied between 47-95%, whereas in cluster IIA varied between 70-84% for highly virulent isolates and single isolate FR 37 was virulent showing 46% disease incidence. Cluster IIB_1 constitutes highly virulent isolates showing 65-100% disease incidence and a virulent isolate FR 5 with 43% disease incidence. Cluster IIB_2 constitutes highly virulent isolates showing 55-80% disease incidence and virulent isolates showing 27-36% disease incidence. Cluster IIB_3 constitutes highly virulent isolates with 54-96% disease incidence and virulent isolates with 38-50% disease incidence. The comparison between the molecular data and bio-chemical data also showed the inevitable relationships between the two. Considering the grouping of the isolates based on fusaric acid production and the cluster generated from the molecular data, it was found that most of the non producers lie in cluster IIB_2 except for the isolates FR5, FR 9, FR 32 and FR 35. Most of the moderate producers of FA were constituted in cluster IIB_3. High producers of fusaric acid locate commonly in two clusters IIB_1 and IIB_3.

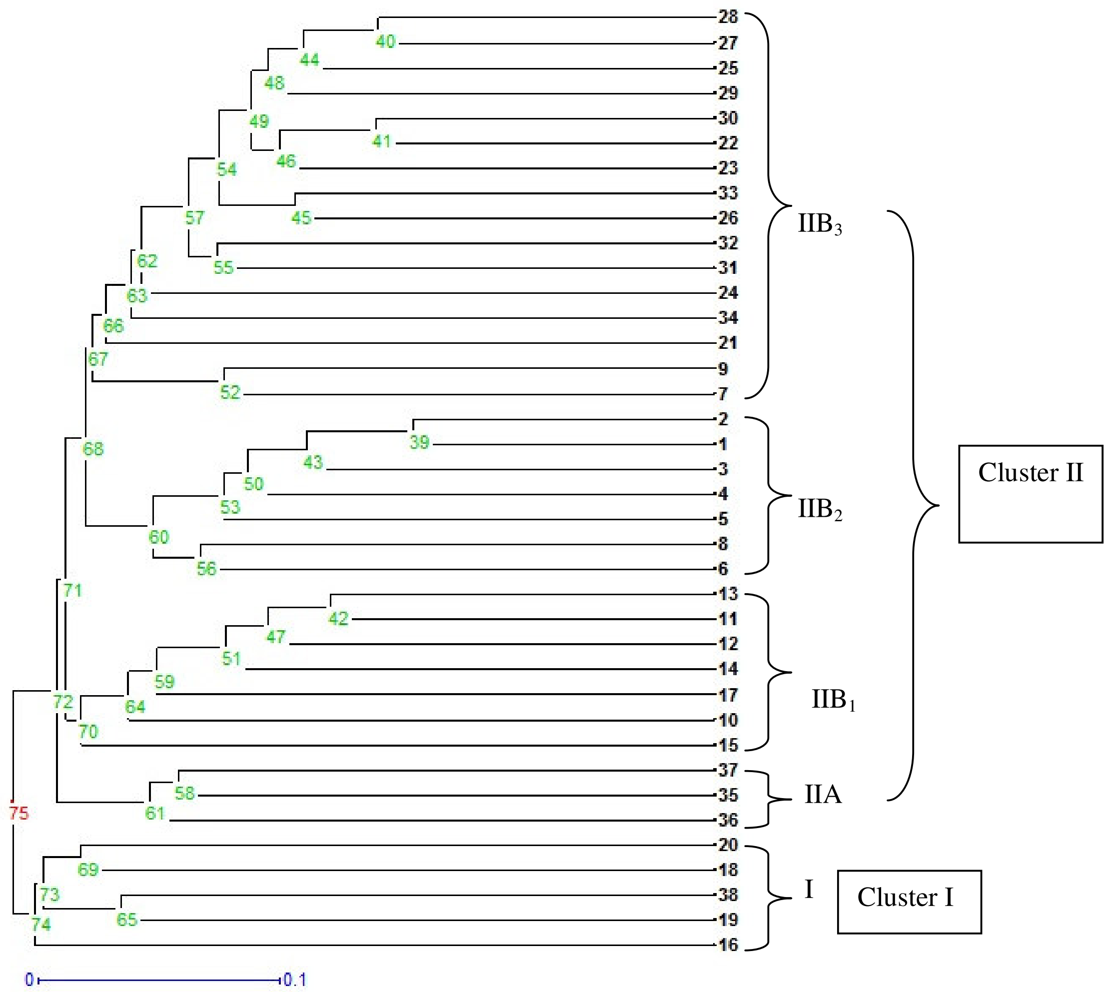


Fig.7 Dendrogram showing molecular diversity among *Fusarium moniliforme* isolates using SSR markers.

Another comparison is the grouping of GA producers and the clusters. It was found that most of the low GA producers were constituted in cluster IIB_3, moderate producers were vastly located in cluster IIB1, IIB2 and IIA but mostly in IIB3. Only four isolates were high producers, out of these two isolates FR 1 and FR 17 were located in cluster IIB_1, IIB_2 respectively and the remaining two isolates FR 18 and FR 20 lie in cluster I.

Table 28: Clustering of *Fusarium moniliforme* isolates based on DARwin 6.0 software program

Cluster No.	Sub Cluster No.	No. of isolates	Isolates
I	I	5	FR 16, FR 19, FR 38, FR 18, FR 20
II	IIA	3	FR 35, FR 36, FR37
	II B_2	7	FR15, FR10, FR17, FR14, FR12, FR11, FR13
	II B_1	7	FR 6, FR 8, FR 5, FR 4, FR 3, FR 1, FR 2
	II B_3	16	FR 7, FR 9, FR 21, FR 34, FR 24, FR 31, FR 32, FR 26, FR 33, FR 23, FR 22, FR 30, FR 29, FR 25, FR 27, FR 28,

Considering the information generated by morphological, biochemical, pathological data and molecular variability, it can be concluded that there studies are informative for assessing the variability. All the methods are inevitable to one another. A single study will not justify the variability study. All the aspects are necessary to draw a fine line in understanding the diversity of the isolates for a region.

The investigations also revealed that variability studies based only on morphological or phenotypic characters are not reliable and molecular techniques are needed to support the phenotypic data. The genetic diversity analysis with SSR markers maximize the study of diverse patterns among the isolates collected from different regions of Punjab and gave an insight to the phenotypic studies supported by the molecular diversity patterns. Understanding variability will help in identifying efficient strategies for the sustainable management of foot rot of basmati.

4.9 Survival of *Fusarium moniliforme* under field conditions

The results of investigations on survival of *Fusarium moniliforme* in soil given in Table 29 revealed that the pathogen doesn’t survive in soil up to next season. The disease incidence of T_6 (naturally infected seeds sown in non-infested soil) was the highest, showing foot rot incidence of 12.39%. It was followed by T_4 (inoculated seeds sown in uninfested soil) showing foot rot incidence of 11.91%. In both the cases, the soil was uninfested and the seeds have the inoculum. The treatment T_1 (healthy seed sown in infested soil) showed the disease incidence of 0.04 % and T_2 (healthy seeds sown in uninfested soil) it was 0.08%.

The studies revealed that soil borne inoculum hardly plays a role have little importance in causing the disease. Considering T_3 and T_4, where the seeds were artificially inoculated were almost at par in terms of disease incidence clearly revealed that only seed

Table 29. Survival of *F. moniliforme* in soil under field conditions

Treatments	Disease incidence (%) (2013)	Disease incidence (%) (2014)
T_1= Healthy seeds on infested soil	0.04	0.01
T_2 =Healthy seeds on uninfested soil	0.08	0.12
T_3=Artificially inoculated seeds on infested soil	11.84	13.50
T_4=Artificially inoculated seeds on uninfested soil	11.91	12.90
T_5 =Naturally infected seeds on infested soil	10.91	9.75
T_6 =Naturally infected seeds on uninfested soil	12.39	10.00
T_7 =Bavistin treated inoculated seeds on infested soil	0.038	0.11
T_8= Bavistin treated inoculated seeds on uninfested soil	0.12	0.15
T_9= Artificially inoculated infected seeds of susceptible cultivar Pusa1121 on uninfested soil	20	25

borne inoculum is responsible for primary infections. If the soil borne inoculums have role in causing the disease then, the field with T_3 might have higher disease incidence than T_4. This indicates that the disease was mainly caused by the inoculums present in the inoculated diseased seeds and the inoculums present in the soil have little to contribute in causing the disease. Similarly, there was not much difference in the disease incidence of T_5 (Naturally infected seeds in infested soil) and T_6 (Naturally infected seeds in uninfested soil) treatments. This shows that the infested and uninfested condition of the soil hardly contributes towards primary infections otherwise T_5 would have surely showed higher disease incidence compared to T_6. These results indicate the primary contribution of infected seeds for infection. The results of the treatments .T_7 and T_8 where seed was treated with Bavistin also supported the findings. The result of the experiment is in conjunct with the findings of Sunder and Satyavir (1998a) and Pannu *et al* (2013). The study also revealed that *Fusarium moniliforme* causing the disease is not a strong soil inhabitant under Punjab conditions. This was also suggested by Nyvall and Kommedahl (1970). Thus, from these findings, we can draw the conclusion that survival of *F. moniliforme* in soil under field condition is not possible till next season and only the infected seeds serve as a primary inoculum.

4.10 Host-range of *F. moniliforme*

Nine weeds collected from rice field were tested against *F. moniliforme*. None of the weeds were found to be the host of foot rot disease. No plants showed symptoms of elongation, stunting or yellowing. All the weed species were free from infection. Thus, the important weeds listed in Table 30 were not found to be the weed host of *F. moniliforme* and plays no role in pathogenesis. However, Carter *et al* (2008) reported survival of *F. fujikuroi* causing bakanae in barnyard and early water grasses and reported to score positive for Koch's

Postulates. But no bakanae symptoms were observed (Carter *et al* 2008). The pathogen has been reported to be isolated from some grasses but no symptoms have been reported yet on those plants. Thus, till date no grass has been reported as alternative host. Further understandings and investigations on the probability of weed as the host of *Fusarium moniliforme* is needed.

From the present study of the survival and host range of the pathogen, a general understanding of the disease cycle has been drawn. From the findings, it is confirmed that the pathogen is seed borne. Kumar *et al* 2015 reported the pathogen to be both externally and internally seed borne. The perusal of the findings also showed that inoculum wasalso present in the soil. However, infection from the soil is of less important as source of inoculum. Primary infection occurs through seed. However, some reports of soil borne inoculum in the form of chlamydospores known at some places of the world. As primary inoculum seeds are more important than the soil borne inoculum under Indian conditions.

Table 30: Investigations on the host range of *F. moniliforme*

Common Name	Scientific Name	Disease incidence (%)
Sanni	*Sphenoclea zeylanica*	0.00
Ammania	*Ammania baccifera*	0.00
Ghuein	*Fimbristylis tenera*	0.00
Madhana	*Dactyloctenuim aegyptiacum*	0.00
Joyweed	*Alternanthra sessilis*	0.00
Chhatri wala motha	*Cyperus iria*	0.00
Narhi gha	*Paspulum distichum*	0.00

When the infected seeds are used for sowing, the emerging seedlings get infected. The infection starts from the nursery and carried to the transplanted over. Secondary infection occurs from the infected seedlings to transplanted ones in the field.

4.11 Screening of Germplasm lines of Basmati against Foot rot disease

The results of screening revealed that out of 134 Basmati germplasm lines, 24 lines were moderately susceptible, 4 lines were susceptible, 4 were highly susceptible, 27 were moderately resistant, 7 lines were resistant, 5 lines were highly resistant. Table 31 shows the percent disease incident of the germplasm lines which were screened under field condition. However, different researchers reported certain genotypes of rice and basmati rice showing resistance against foot rot from different parts of the world (Bagga and Kumar 2000, Saremi *et al* 2008, Ma *et al* 2008, Iqbal *et al* 2011, Ghazanfar *et al* 2013, Pannu *et al* 2013).
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Table 31: Evaluation of germplasm lines of basmati rice against foot rot disease under field conditions

Sl. No	Entry	Designation	Nursery		Transplanted	
			Disease Incidence	Host Response	Disease incidence	Host Response
1	RYT-BT-1-1	6001*	50	MS	50	MS
2	RYT-BT-1-2	6002	38	MR	50	MS
3	RYT-BT-1-3	6004	50	MS	50	MS
4	RYT-BT-1-4	RYT-BT-6	50	MS	40	MR
5	RYT-BT-1-5	RYT-BT-10	25	MR	30	MR
6	RYT-BT-1-6	RYT-BT-15	45	MS	40	MS
7	RYT-BT-1-7	RYT-BT-16	10	HR	10	HR
8	RYT-BT-1-8	AVT-IBT-2101	35	MR	30	MR
9	RYT-BT-1-9	AVT-IBT-2103	40	MR	40	MR
10	RYT-BT-1-10	AVT-IBT-2104	25	MR	30	MR
11	RYT-BT-1-11	AVT-IBT-2106	14	R	20	R
12	RYT-BT-1-12	CR-2007	14	R	20	R
13	RYT-BT-1-13	Pusa Punjab Bas-1509	60	S	65	S
14	RYT-BT-1-14	Pusa Bas 1121	80	HS	85	HS
15	RYT-BT-1-15	Punjab Bas 2	50	MS	50	MS
16	RYT-BT-1-16	Bas 370	70	S	70	S
17	RYT-BT-1-17	Bas 386	70	S	65	S
18	RYT-BT-1-18	Punjab Basmati-3	88	HS	80	HS
19	RYT-BT-1-19	Sharbati	40	MR	35	MR
20	RYT-BT-1-20	PB Mehak-1	28	MR	30	MR
21	RYT-BT-1-21	Dewe Gowda	50	MS	60	MS
22	RYT-BT-2-1	6291-1	67	S	70	S
23	RYT-BT-2-2	6295-2	40	MR	40	MR
24	RYT-BT-2-3	6297-1	57	MS	40	MR
25	RYT-BT-2-4	6298-3	10	HR	10	HR
26	RYT-BT-2-5	6305-2	30	HR	10	HR
27	RYT-BT-2-6	6306-1	90	HS	90	HS
28	RYT-BT-2-7	6307-2	50	MS	60	MS
29	RYT-BT-2-8	6308-2	47	MS	30	MR
30	RYT-BT-2-9	6328-2	37	MR	50	MS
31	RYT-BT-2-10	6329	90	HS	90	HS
32	RYT-BT-2-11	6330	10	HR	10	HR
33	RYT-BT-2-12	6331-1	33	MS	50	MS
34	RYT-BT-2-13	6396	40	MR	50	MS
35	RYT-BT-2-14		56	MS	40	MR
36	RYT-BT-2-15	6670-1	35	MR	40	MR

37	IYT-BT-101	6283-1	40	MR	40	MR
38	IYT-BT-102	6284-1	67	S	60	MS
39	IYT-BT-103	6285-2	65	S	60	MS
40	IYT-BT-104	6288	55	MS	40	MS
41	IYT-BT-105	6289-2	28	MR	20	R
42	IYT-BT-106	6290-2	43	MR	50	MS
43	IYT-BT-107	6292-1	30	MR	50	MS
44	IYT-BT-108	6294	45	MS	50	MS
45	IYT-BT-109	6300-1	20	R	20	R
46	IYT-BT-110	6301-2	56	MS	30	MR
47	IYT-BT-111	6302-1	20	R	20	R
48	IYT-BT-112	6303-3	50	MS	30	MR
49	IYT-BT-113	6304-1	50	MS	50	MS
50	IYT-BT-114	6306-4	38	MR	40	MR
51	IYT-BT-115	6308-1	30	MR	20	R
52	IYT-BT-116	6310-1	50	MS	60	S
53	IYT-BT-117	6310-3	30	MR	20	R
54	IYT-BT-118	6314-2	67	S	40	MR
55	IYT-BT-119	6315-2	50	MS	30	MR
56	IYT-BT-120	6318-3	60	MS	50	MS
57	IYT-BT-121	6321-2	55	MS	60	MS
58	IYT-BT-122	6322	60	MS	50	MS
59	IYT-BT-123	6338-1	45	MS	50	MS
60	IYT-BT-124	6339	38	MR	40	MR
61	IYT-BT-125	6342-2	25	MR	30	MR
62	IYT-BT-126	6344-2	45	MS	30	MR
63	IYT-BT-127	6346	50	MS	40	MR
64	IYT-BT-128	6350-2	55	MS	40	MR
65	IYT-BT-129	6356-1	30	MR	30	MR
66	IYT-BT-130	6357-2	35	MR	30	MR
67	IYT-BT-131	6361	47	MS	30	MR
68	IYT-BT-132	6362-2	30	R	20	R
69	IYT-BT-133	6366	37	MR	40	MR
70	IYT-BT-134	6372-1	50	MS	40	MR
71	IYT-BT-135	6377-2	40	MR	50	MS
72	IYT-BT-136	6385	67	S	60	MS
73	IYT-BT-137	6386-1	60	MS	70	S
74	IYT-BT-138	6387	70	S	70	S
75	IYT-BT-139	6388-4	30	MR	30	MR
76	IYT-BT-140	6389-1	57	MS	60	MS
77	IYT-BT-141	6393	20	R	10	HR

78	IYT-BT-142	6397	57	MS	40	MR
79	IYT-BT-143	6400-1	40	MR	30	MR
80	IYT-BT-144	6401-2	50	MS	60	MS
81	IYT-BT-145	6405-1	30	MR	40	MR
82	IYT-BT-146	6411	30	MR	30	MR
83	IYT-BT-147	6447	45	MS	30	MR
84	IYT-BT-148		20	R	20	R
85	IYT-BT-149	6451-3	50	MS	30	MR
86	IYT-BT-150	6456-1	60	MS	20	R
87	IYT-BT-151	6458-1	60	MS	80	S
88	IYT-BT-152	6459-1	50	MS	50	MS
89	IYT-BT-153	6461-2	60	MS	60	MS
90	IYT-BT-154	6463	40	MR	60	MS
91	IYT-BT-155	6465	25	MR	50	MS
92	IYT-BT-156	6466-1	33	MR	40	MR
93	IYT-BT-157	6482-2	10	HR	30	MR
94	IYT-BT-158	6485	30	MR	50	MS
95	IYT-BT-159	6488-2	30	MR	30	MR
96	IYT-BT-160	6489-3	60	MS	40	MR
97	IYT-BT-161	6490-2	30	MR	40	MR
98	IYT-BT-162	6500	67	S	40	MR
99	IYT-BT-163	6508	33	MR	20	R
100	IYT-BT-164	6521-1	30	MR	50	MS
101	IYT-BT-165	6525-2	50	MS	60	MS
102	IYT-BT-166	6533-2	80	S	70	S
103	IYT-BT-167	6537-4	20	R	75	S
104	IYT-BT-168	6538-2	30	MR	75	S
105	IYT-BT-169	6541-1	60	MS	46	MS
106	IYT-BT-170	6549-1	40	MR	60	MS
107	IYT-BT-171	6552-2	100	HS	100	HS
108	IYT-BT-172	6553-2	60	MS	40	MR
109	IYT-BT-173	6554-2	40	MR	55	MS
110	IYT-BT-174	6594	60	MS	50	MS
111	IYT-BT-175	6612	10	HR	15	R
112	IYT-BT-176	6638	20	R	60	MS
113	IYT-BT-177	6653	30	MR	45	MS
114	IYT-BT-178	6662	30	MR	39	MR
115	IYT-BT-179	6665	60	MS	33	MR
116	IYT-BT-180	6669	30	MR	40	MR
117	32799 Bulk	PAU 3245-42	20	R	16	R

118	32800-Bulk	PAU 3245-43	40	MR	45	MS
119	32804-Bulk	PAU 3245-47	10	HR	10	HR
120	32807-Bulk	PAU 3245-50	10	HR	44	MS
121	32808-Bulk	PAU 3245-51	21	MR	20	R
122	GSK-27	IR 74718-147-1-1-3	33	MR	20	R
123	GSK-65	IR 75478-55-3	50	MS	20	R
124	GSK-95	IR 75479-46-1-2	46	MS	30	MR
125	GSK-98	IR 75479-59-3-3	57	MS	40	MR
126	GSK-133	IR 75482-140-2-3	60	MS	80	S
127	GSK-139	IR 75483-33-3-2	50	MS	70	S
128	GSK-140	IR 75483-50-3-1	13	R	10	HR
129	GSK-188	IR 75490-337-2-1	38	MR	10	HR
130	GSK-194	IR 71735-6-3-3	67	S	20	R
131	GSK-196	IR 44699-21-1-3-4	39	MR	20	R
132	2484 ctk		80	S	50	MS
133	2485 Ctk		100	HS	100	HS
134	2486 Ctk		30	MR	40	MR

CHAPTER V

SUMMARY

Foot rot (bakanae) caused by *Fusarium moniliforme* is one of the major fungal diseases of rice. In India, bakanae disease incidence has been increasing considerably and reported to cause substantial losses in grain yield during recent years. The cultivars of basmati rice are more susceptible to this disease. In the present study, pathogenic, bio-chemical, molecular and genetic diversity among different populations of *F. moniliforme* (syn. *F. verticilloides*) has been investigated.

Investigations on cultural characteristics of *F. moniliforme* on different media *viz*., Potato dextrose Agar (PDA), Soil Extract Agar (SEA) and Spezieller Nahrstoffarmer Agar (SNA) showed that color of different isolates varied from whitish to pinkish on the surface. Higher intensity of pigments was observed on PDA as compared to SEA. The pathogen culture on SEA media mostly showed white to milky white color, however, on PDA the culture produced visibly distinct pigments at the centre which diffused out to the periphery. Only some of the isolates produced pigment on the reverse. The growth was dense and fluffy for both PDA and SEA, moderate and not fluffy on SNA. The isolates also showed variation in colony diameter and growth rate on all the three media. The variability among the isolates has been observed on the same media and also between different media. The best growth was observed on PDA, followed by SEA and SNA. The growth rate varied between 12.28-25.86-mm/ 48 hrs on PDA, between 9.09-19.59 mm/ 48 hrs on SEA and between 5.97- 16.44 mm/ 48hrs on SNA media. Statistical analysis through Duncan Multiple Range test indicated that most of the isolates were significantly different (P=0.05). The studies revealed that different culture media influenced the growth and pigmentation of the culture.

Morphological studies of micro-conidia and macro-conidia showed that the size of the spores varied among the 38 isolates in the range of (6.8-9.8) X (2.9-6.0) µm for micro-conidia with no septa and (24.23-62.97) X (2.90-4.27) µm for macro-conidia with 3-5 septa. Micro-conidia were oval/ ovoid in shape with flattened base, usually 0-septate and produced in large numbers. Macro-conidia were septate, slightly curved and were canoe or needle-like appearance under the microscope with basal and apical cell. The basal cell appeared as distinctly notched or barely notched and apical cell as hooked. Chains of micro-conidia were formed on water agar with KCl and chlamydospores were absent on all the isolates. On the basis of morphological and cultural characters the isolates were identified as *F. moniliforme* (syn. *F. verticilloides*) or *F. proliferatum* (Leslie and Summerell 2006). The morphological identification was further confirmed by using species specific primers. DNA from 38 isolates was amplified with the set of primers VERT1 and VERT2 and 35 out of 38 isolates gave a single amplicon of 800bp. This confirmed that the isolates belonged to *F. moniliforme* (syn. *F*

verticilloides). None of the isolates showed bands for the primers specific for *F. proliferatum*. The study confirms that identification based on cultural and morphological features only is not reliable, thus the identification of species using species specific primers is required.

Elongated and slender seedlings produced after 15 days of sowing artificially inoculated seed with spore suspension of *Fusarium moniliforme*. Diseased seedlings were pale yellow and often visibly taller than the healthy rice plants. Some of the seedlings remained stunted as compared to the normal plants. The elongated seedlings emerged from the infected seeds and died after few days. Seedlings often progressively die from the seedling stage through to maturity. Noticeable pink to white fungal growth and profuse sporulation of the fungus were observed on lower parts of the stems of diseased plants. All the 38 isolates were found to be pathogenic.

All the isolates showed variable production of gibberellic acid (GA) and fusaric acid (FA). Fusaric acid was produced by 30 isolates out of 38 and GA has been produced by all 38 isolates. Fusaric acid was produced in the range of 0.80-6.40 µg/ml. Only the isolate FR 1 produced FA concentration below 1 µg/ml. It produced the lowest concentration of FA among the 30 isolates whereas FR 6 produced the highest. On the basis of quantitative estimation of FA, the isolates can be categorized as none producers, low producers (<1 µg/ml), moderate producers (1-3 µg/ml) and high producers (>3 µg/ml). Eight isolates (FR 5, 9, 10, 11, 12, 13, 32, 35) failed to produce FA, single isolate FR1 produced <1 µg/ml, 19 isolates (FR2, 3, 7, 14, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29,30, 36, 37) were termed as moderate producers and 10 isolates (FR 4, 6, 8, 15, 16, 25, 31, 33, 34, 38) as high producers.

Quantitative estimation of GA in the samples was determined spectrophotometrically. All the 38 isolates showed positive results for the production of GA by TLC. These isolates showed variable production of GA in the range of 3.23-23.4 µg/ ml. On the basis of gibberellin production, fungal isolates were classified as low producer (<10 µg/ ml), moderate producer (11-20 µg/ ml) and high producer (>20 µg/ ml) (Table 25). Nine isolates (6, 8, (15-16), 22, 25, 31, 34, 38) were low producers, 25 isolates FR (2-5), 7, (9 -14), 19, 21, (23-24), (26-30), (32-33), (35-37) were moderate producers and four isolates FR 1, (17-18) and 20 were found as high producers. A close analysis showed that most of the isolates produced 10-20µg/ml GA and therefore, most of the isolates belong to moderate producers. Statistical analysis showed that the production of FA significantly correlates positively with the number of stunted plants in pathogenicity test and GA production positively correlated with the number of elongated plants. Positive correlation between GA production and elongation symptoms confirmed the role of GA causing foolish seedling type symptoms. Similarly, FA production and the number of stunted plants also showed positive correlation and this revealed the involvement of fusaric acid in causing stunting type of symptoms in foot rot infected plants. Some isolates (FR 5, FR 9, FR 10, FR 12 and FR 13) didn't produce FA.

However, these produced stunting of infected seedlings. Presence of both the factors or of either of them resulted in diseased seedlings. Comparing the contribution of GA and FA in the disease incidence, it was observed that the role of GA is more profound.

Fifty primers were screened to assess the genetic diversity among the 38 isolates of *Fusarium moniliforme.* Among the 50 primer pairs designed, 27 pairs (54%) were able to amplify 1or more alleles of diverse *F. verticillioides* isolates. Eleven primers (1H02, 2H17, 2H15, 3H02, 3H19, 5H09, 9H05, 10H09, 10H01 and 11H01) amplified more than two alleles. A total of 102 alleles were detected by 27 primers. The number of alleles generated by each primer varied from 1 to 10. Polymorphic Information Content (PIC) varied from 0.10-0.89 with an average of 0.46. These markers are highly informative as they indicated high polymorphism. The PIC value can be looked as the measurement of usefulness of each primer in distinguishing one individual from another. The genetic dissimilarity index ranged from 25% to 75%.

The dendrogram generated from the molecular data showed grouping of 38 isolates into two distinct clusters. Cluster I is smaller than Cluster II consisting of five isolates (FR20, FR 18, FR 38, FR19 and FR16). Cluster II is the largest and comprised of 33 isolates, constituting of two sub groups: IIA and IIB. Subcluster IIA constitutes three isolates and it is smaller than the subcluster IIB. Subcluster IIB consists of another three subclusters $IIB_{1,}$ IIB_2 constituting of seven isolates each and IIB_3 with sixteen isolates.

The studies revealed that morphological grouping, pathogenicity data and bio-chemical data showed some resemblance to clustering based on molecular data. However, the individual group based on phenotypic and bio-chemical did not locate distinctly in a particular cluster. Majority of the isolates of each group locate to a particular cluster but not all. A few of them were found to locate in another group. Considering the information generated by morphological, biochemical, pathological and molecular variability, it can be concluded that all the studies are informative and inevitable for studying the variability and all the isolates showed variability in different aspects under study. It has been observed that study of one aspect may not justify the diversity. All the aspects are necessary to draw a fine line in understanding the diversity of the isolates for a region. The results also revealed that variability studies based only on morphological or phenotypic characters are not reliable and hence, molecular techniques are needed to support the phenotypic data. The genetic diversity analysis with Simple Sequence Repeats (SSR) markers helped to study the diverse patterns among the isolates collected from different regions of Punjab and gave an insight to the phenotypic studies supported by the molecular data. Understanding variability will help in identifying efficient strategies for the sustainable management of foot rot of basmati.

Investigations on survival of *Fusarium moniliforme* in soil revealed that the pathogen doesn’t survive in soil up to next season. This indicates that soil borne inoculum have little

role in causing the primary infection. These results revealed that seed borne inoculum is primary source of infection. It has been observed that pathogen doesn’t produce chlamydospores which can survive in soil for longer period of time and this limits its survival in soil. Artificial inoculations of nine weeds revealed that none was found host of foot rot disease.

Out of 134 Basmati germplasm lines screened under field conditions, 24 lines were moderately susceptible, 4 lines were susceptible, 4 were highly susceptible, 27 were categorized as moderately resistant, 7 lines were resistant, 5 lines were found highly resistant.

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RESEARCH ARTICLES

SR. NO.	PUBLICATION	JOURNAL	IMPACT FACTOR	NAAS RATING	STATUS
1.	Variability in production of gibberellic acid and fusaric acid by *Fusarium moniliforme* and their relationship.	Journal of Plant Pathology	1.933	7.09	Accepted
2.	Investigations on survival and host range of F*usarium moniliforme* Sheld. causing foot rot disease of basmati.	Environment and Ecology	0.013	4.09	Accepted
3.	Cultural, morphological and molecular variability of *Fusarium moniliforme* Sheld. Causing bakanae disease of basmati in Punjab.	Indian Journal of Microbiology	1.143	6.90	Submitted